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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00841-12 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methods for Comparing and Analyzing Data from Several Complex Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
Others:	H.J. Hoffman	Chief, Epidemiology	OD, EB, NIDCD
	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
	Y.J. Lee	Chief	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

BB, CTS, DCPC, NCI (B. Graubard); BRB, NCI (E. Korn)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will develop statistical methods for the analysis of data from complex sample surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). The research concentrates on ways to test hypotheses about the coefficients that derive from multiple linear regression of survey data. Existing methods which use Wald test statistics are compared to newly developed approaches based upon Bonferroni t-statistics and jackknifed Wald statistics. These regression methods have been applied to the NHANES data sets to determine if they can be used to provide ways to analyze the complex relationships of growth and nutrition.

Survey statistical methods proper for the National Health Interview Survey (NHIS) have begun. Data sets from the Child Health Supplement of NHIS 1988 will also be examined.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00850-17 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS (if any)

Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson); Intramural Research, Developmental Neurology Branch (D. Hirtz)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study, which began in 1974, is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants were followed through six years of age.

The Biometry and Mathematical Statistics Branch served as a data center for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency.

The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. During this year a comparison of binding (Sephadex, HBABA) results were compared to bilirubin levels and the six year outcome of the child.

The results of the newborn data were published in a supplement to Pediatrics in February 1985. Six-year follow-up data was published in Pediatrics, April, 1990. Intelligence at six years in relation to neonatal bilirubin was published in Pediatrics, June, 1991.

003

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00854-09 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of MCH Data from the National Longitudinal Youth Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); Ohio State University (F. Mott)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Branch's primary goal in this project is to collect, analyze, and publish data based on a series of interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. This survey has been collecting data on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The Biometry & Mathematical Statistics Branch (BMSB) began collecting data on pregnancy outcome and the first year of life of the child in 1983. With this data base, analysis of trends over time in the maternal and child health will be done. We will concentrate on analyzing prenatal care and birth weight versus working and non-working mothers, well baby care including immunizations by socio-economic characteristics.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00855-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methods for the Common Odds Ratio of a Number of Contingency Tables

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI: K.F. Yu Mathematical Statistician BMSB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.15	0.15	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will develop statistical methods for estimating the standardized risk difference and the common odds ratio for a number of contingency tables. The optimality properties of the developed methodology will be investigated and established. The likelihood equations are to be derived and the weighted least squares, the weighted total, the maximum likelihood and the conditional maximum likelihood and the conditional maximum likelihood methodologies are to be examined. The study has been extended to do comparison of a number of contingency tables in the form of hypothesis testing. All these methodologies have wide applications to epidemiological studies, biomedical studies, genetics and other disciplines.

007

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00856-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods for a Mixture of Subpopulations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
Others:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
	H.J. Hoffman	Chief	EB, OD, NIDCD

COOPERATING UNITS *(if any)*

Norway National Institute of Public Health (L.S. Bakketeig); National Center for Health Statistics (C.J. Krulewitch); Chung Yuan Christian University (M.S. Yang)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will examine the intrinsic difficulty of estimating subpopulation characteristics when the identities of the observations are not completely known. Hard clustering and fuzzy clustering techniques are investigated. This study will develop estimation of methodologies for the subpopulation characteristics. The new methodologies will alleviate some of the intrinsic difficulties for a heterogeneous mixture of subpopulations. Applications will be made to study the issues concerning biological heterogeneity and measurement error in the birth weight, gestational age and perinatal mortality. This study will also be applied to the investigation of intrauterine growth and pregnancy outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00857-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodological Research in Mathematical Statistics and Biostatistics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Y.J. Lee	Chief	BMSB, DESPR, NICHD
Others:	J. Troendle	Staff Fellow	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Hankook University of Foreign Studies (T. Park)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methodological research in statistics is motivated often by interest in methodological/theoretical statistical questions. Consulting and collaborative projects also motivate the research because existing methods and theories may be inadequate to handle their data analysis or study design.

Dr. Lee is now completing a paper on missing data analysis. And he is also finishing up a paper on confidence interval for the difference in survival rates of two populations. Methodological research in designing a study where incomplete data are expected is ongoing. A simulation evaluation of competing methods has been completed in collaboration with Dr. Park. Methodological research is motivated by general statistical and biostatistical interest as well as needs for biostatistical collaborations with subject matter scientists. Dr. Lee has undertaken a methodological research in regression analysis of censored data. Drs. Lee and Troendle are looking into a new class of change point problems that have been generated from a consulting project.

Dr. Troendle is continuing to work on resampling based methods of multiple testing. This research was motivated by the DIEP study, in which 55 simultaneous hypothesis tests were conducted, and has been found useful in analyzing MRI results. Recently, Dr. Troendle has finished a manuscript analyzing the practical options available to the data analyst when using recursive partitioning. This method of classification was found useful in a study to find the best anthropometric surrogate birth weight.

011

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00859-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Meta-Analytic Methods

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Rebecca DerSimonian Mathematical Statistician BMSB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Biometry and Mathematical Statistics Branch, DESPR

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.40	0.40	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This ongoing project consists of several components:

One component considers a fixed and a random effects model for combining data from a series of comparative trials. The study evaluates the performance of each method under various heterogeneity assumptions and with several scales of measurement, including the risk difference and the odds ratio as measures of effect.

Another component develops and compares parametric and non-parametric tests to assess the assumption of homogeneity of effects in data from a series of trials. This component considers data from Gaussian, binomial, and poisson sampling distributions.

A third component extends and adapts the meta-analytic methods appropriate for clinical trials to combining the evidence from a series of epidemiological studies (both case-control and cohort designs). Partial results from this component appear in the Journal of Clinical Epidemiology.

A general application of the methods of this project appears in Clinics of Applied Nutrition.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00862-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Contingency Tables with Missing Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rebecca DerSimonian	Mathematical Statistician	BMSB, DESPR, NICHD
	Stuart Baker	Mathematical Statistician	BB, DCPC, NCI

COOPERATING UNITS *(if any)*

The Biostatistics Center, George Washington University (W.F. Rosenberger)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.10	0.10	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreducec type. Do not exceed the space provided.)*

This study develops closed-form maximum likelihood estimates for data from contingency tables with missing values on some or all variables. We derive the estimates for two-way contingency tables via loglinear models that are easy to specify and that generalize readily to multi-way tables with missing data on some or all variables. The loglinear models we propose have the advantage of providing information about the missing-data mechanism and ascertaining how assumptions about the missing-data mechanism affect inference. Results of the method for two-way contingency tables appear in Statistics in Medicine.

016

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00863-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Analysis for Vaccine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Developmental & Molecular Immunity, NICHD (J Robbins)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is a continuous collaboration with the Laboratory of Developmental and Molecular Immunity (LDMD) in vaccine development. LDMI is developing vaccines against such diseases as cholera, meningitis, shigellosis, tuberculosis and bacteremia.

The Biometry and Mathematical Statistics Branch analyzes antibody titers from animal and human sera, participates in protocol preparation, clinical trial design and manuscript preparation. Various statistical tests, such as, paired and unpaired t test, Fisher's Exact test, Pearsons correlation and other tests, are used when appropriate.

018

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00865-02 BB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Linear Models that Include Transformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Rebecca DerSimonian Mathematical Statistician BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Emory University School of Public Health (W.D. Flanders); Centers for Disease Control (D.S. Freedman)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In ordinary linear regression analysis, we must often transform the dependent variable to satisfy the usual statistical assumptions of normality, variance stability, and linearity. Use of transformations, however, complicates interpretations of model parameters. In this project, we derive simple interpretations of linear models that include transformations or interaction terms. These derivations do not replace more traditional presentations but complement and enhance them as an aid to understanding results. A key advantage is that they allow the presentation of results on the original, untransformed scale that is familiar to clinicians, rather than on transformed scale that involves logarithms or power transformations.

020

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01600-09 CBMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Basis of T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.E. Samelson, Medical Officer (Research), CBMB, NICHD

Others: J. Donovan, IRTA, CBMB
Y. Ota, Visiting Fellow, CBMB
M.L. Egerton, Visiting Fellow, CBMB
R.L. Wange, IRTA, CBMB
N. Isakov, Adjunct Scientist, CBMB
S. Meyers, EBON, CBMB

COOPERATING UNITS *(if any)*

Laboratory of Immunoregulation, NIAID, NIH (D.I. Cohen); NCI (A. Singer); Naval Medical Research Institute (C. June); Washington University (A. Shaw)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.375	3.875	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Engagement of the multicomponent antigen receptor in T cells (TCR) results in rapid activation of a protein tyrosine kinase pathway. To fully understand the function of this pathway, the kinase and its substrates must be characterized. We have previously shown that the protein tyrosine kinase $p60^{fyn}$ is associated with the TCR. Current studies demonstrate specific sites within the kinase molecule responsible for this interaction. Another candidate for a TCR-protein tyrosine kinase is ZAP, a protein that binds to the activated TCR. Recent work from our laboratory demonstrates that tandem SH2 domains in this kinase are responsible for its interaction with TCR subunits following their tyrosine phosphorylation. One substrate for tyrosine kinases activated by the TCR is the 100kD valosin-containing protein (VCP). As a first step in understanding the effect of tyrosine phosphorylation on this protein, sites of phosphorylation have been mapped to the C-terminus of VCP. The protein has also been shown to have ATPase activity, as predicted from sequence analysis. Tyrosine phosphorylation of VCP has no effect on ATPase activity. Abnormal tyrosine phosphorylation of substrates has been detected in T cells that are infected with human immunodeficiency virus (HIV). One of the prominent substrates in this system is the protein kinase, p34cdc2, a regulator of mitosis. Accumulation of tyrosine phosphate on this enzyme correlates with the cell cycle arrest at G2/M that can be observed with HIV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01601-08 CBMB

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Aspects of the Regulation of the Human Transferrin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.B. Harford, Senior Investigator, CBMB, NICHD
J. Basilion, IRTA, CBMB, NICHD
R. Binder, NRC Fellow, CBMB, NICHD
J. Horowitz, IRTA, CBMB, NICHD
R.D. Klausner, Chief, CBMB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
		0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT INCORPORATED INTO NEW PROJECT ENTITLED "INTRACELLULAR IRON METABOLISM,"
PROJECT NUMBER CBMB Z01 HD 01610-01

PHS 6040 (Rev. 5/92)

SENT BY:BLDG 1ST RM. 101 :10-12-93 ; 2:05PM ; NIH CBMB- 3014020105:# 2/ 2

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01602-09 CBMB

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intracellular Iron Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD

Co-PI: T.A. Rouault, Senior Investigator, CBMB, NICHD

Others: J. Basilion, Staff Fellow, CBMB
R. Binder, Bio-Tech Fellow, CBMB
J. Chin, Senior Staff Fellow, CBMB
D. Haile, Medical Staff Fellow, CBMB
J. Harford, Expert, CBMB
J. Horowitz, Senior Staff Fellow, CBMB
K. Iwai, Adjunct Scientist CBMB
C. Massinople, EBON, CBMB
C. Philpott, IRTA, CBMB
C. Tang, IRTA, CBMB

COOPERATING UNITS *(if any)*

Scripps Research Institute, La Jolla, CA (D. Stout)
American Red Cross, Rockville, MD (W. Burgess)
University of Wisconsin, Milwaukee, WI (H. Beinert, C. Kennedy)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.55	7.75	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at understanding the molecular basis of intracellular iron metabolism. The cis and trans elements mediating the iron-dependent alterations in abundance of ferritin and the transferrin receptor have been identified and characterized in previous years in this laboratory. Iron-responsive elements (IREs) are RNA stem-loops found in the 5' end of ferritin mRNA and the 3' end of transferrin receptor mRNA. We have cloned, expressed, and characterized an essential iron-sensing protein, the iron-responsive element binding protein (IRE-BP). The IRE-BP binds IRE's when iron levels are depleted, resulting in the inhibition of translation of ferritin mRNA and prolongation of the half-life of the transferrin receptor mRNA. The IRE-BP is 30% identical in amino acid sequence to aconitase, a mitochondrial Krebs cycle enzyme. Mitochondrial aconitase has previously been purified and crystallized and all active site residues are identical in the two proteins. Three of the active site residues are cysteines that ligate an iron-sulfur cluster that has the relatively unusual feature of containing a labile fourth iron. The IRE-BP has aconitase activity, and in vitro manipulations of iron result in changes in RNA binding. In vivo, reciprocal regulation of aconitase activity and RNA binding can be seen when the IRE-BP is over-expressed in a stable cell line. Regulation of RNA binding activity involves a transition from a [4Fe-4S] that does not bind RNA and is an active aconitase to a form that loses iron and aconitase activity. Controlled degradation of the iron-sulfur cluster reveals that the apoprotein is the physiologically relevant form of the protein in iron-depleted cells. Thus, the cluster appears to be undergoing constant disassembly, and reassembly depends on the presence of sufficient iron pools.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 01605-06 CBMB

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD

Others: F. Letourneur, Adjunct Scientist, CBMB, NICHD
I. Engel, IRTA, CBMB, NICHD
T. Ottenhoff, Special Volunteer, CBMB, NICHD

COOPERATING UNITS *(if any)*

Andrey Shaw, Washington University, St. Louis, MO
Don Wiley, Harvard University, Cambridge, MA
Andrew McMicheal, Oxford University, Oxford, England

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this group are to identify the structural components of the T cell antigen receptor complex, to attempt to understand the structure of that complex and to relate that structure to how the complex assembles, is transported throughout the endomembrane system and expressed on the cell surface, and, finally, how it actually functions to activate the T lymphocyte. This group has used gene transfection and mutagenesis studies to address and identify the subunit interactions within the eight-chain receptor complex. These studies have led to a model for the structure of the T cell receptor. One of the major themes of this groups has been the identification of specific molecular motifs that are responsible for the assembly, intracellular targeting, and function of the receptor. Major advances have been in the identification of these motifs, and particularly in the identification and characterization of the structural motif responsible for the activation of T cells, and the demonstration that this motif is a repetitive one that is responsible not only for T cell activation, but for B cell activation and for the activation of other cells of the inflammatory system such as the cells responsible for the allergic response, mass cells and basophils. Finally, this group has successfully developed a molecular engineering approach to solve the vexing problem of how to produce large amounts of purified, functional T cell receptor antigen recognition heterodimers. Accomplishing this task utilized the information gathered over the pst several years in this laboratory on the structural motifs involved in both assembly and intracellular targeting. The approach developed appears to be a generally applicable one and the group has successfully expressed multiple human and murine T cell receptors. The group is now in the process of producing milligram quantities of the purified soluble receptor heterodimer for x-ray crystallography studies with Dr. Don Wiley at Harvard University.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 HD 01606-05 CBMB

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biology of Early Organelles of the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD

Others: J. Donaldson, Senior Staff Fellow
D. Finazzi, Special Volunteer, CBMB
A. Finegold, Guest Researcher, CBMB
F. Hochstenbach, Visiting Fellow, CBMB
V. Hsu, Senior Staff Fellow, CBMB
P. Peters, Visiting Fellow, CBMB
S. Teal, Guest Researcher, CBMB
L. Yuan, Chemist, CBMB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.2		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The central vacuolar system of eukaryotic cells is an interlocking membrane system composed of distinct organelles (including ER, Golgi apparatus, secretory vesicles, secretory granules, plasma membrane, endosomes and lysosomes) and selective transport pathways. Proper functioning of this system is vital for regulating surface expression and secretion of proteins. Using the fungal metabolite brefeldin A (BFA), which dramatically alters both the distribution and flow of membrane through the central vacuolar system, this group has focused on how organelles maintain their structure and identity, and how membrane trafficking between organelles is regulated. These questions have been directed to three different areas: (1) Understanding the biochemical basis of BFA action. We have found that BFA appears to affect the Golgi apparatus by acting on specific membrane targets that regulate the assembly of cytosolic coat proteins onto the cytoplasmic face of this organelle. Altered interaction of the coats with the organelle leads to organelle disassembly, tubule formation and mixing of organelle components within a defined "homotypic" membrane system. The biochemistry of two BFA sensitive coat proteins, β-COP and ARF are under investigation. (2) The biology and biochemistry of ARF family proteins. The recognition that the activation of the family of small GTP binding proteins called ARF's is most likely the target for BFA has led us to an extensive study of the biochemistry and cell biology of individual ARF family proteins using *in vitro* reconstitution of biochemical assays, mutagenesis and expression of wild type and mutant ARF proteins in cells. (3) Characterization of the distribution and flow of membrane within the vacuolar system. We have found that BFA causes the various compartments of the vacuolar system to collapse into new steady states which produce isolated but functioning new organelle units. Traffic within but not between these units continues in the presence of BFA. In summary, our studies with BFA provide new insights about the properties of sets of organelle-specific coat proteins and presents a framework for relating the biochemical regulation of membrane transport to the structure and maintenance of organelles. We have continued studies on the mechanisms of retention of proteins within the early organelles of the vacuolar system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01607-03 CBMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Regulation of the Fate of Newly Synthesized Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.S. Bonifacino Visiting Scientist CBMB, NICHD

Others: M.D. Delahunty IRTA Fellow CBMB, NICHD

M.S. Marks NRSA/IRTA Fellow CBMB, NICHD

J. Humphrey Medical Staff Fellow CBMB, NICHD

E. Deignan HHMI-NIH Scholar CBMB, NICHD

C. Bonnerot Special Volunteer CBMB, NICHD

COOPERATING UNITS *(if any)*

R. Germain, LI, NIAID

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.7	4.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work in this group has focused on the molecular and cellular mechanisms that govern the intracellular localization and trafficking of newly synthesized proteins in the central vacuolar system. Specific areas of interest have included the relationship between protein assembly and transport from the endoplasmic reticulum (ER) and the mechanisms of protein localization to the *trans*-Golgi network (TGN).

Studies of protein assembly in the ER were done using the multisubunit class II molecules of the major histocompatibility complex (MHC) as a model system. Class II MHC molecules are composed of two polymorphic chains (α and β), that associate in the ER with a third non-polymorphic chain known as the invariant chain (I). Our studies have shown that various incompletely assembled forms of class II MHC molecules are largely retained in the ER, where they bind non-covalently to the immunoglobulin heavy chain binding protein, BiP. In addition, the incomplete complexes have been found to form large homotypic aggregates. On the basis of these observations, we have proposed that interaction with ER resident proteins such as BiP and aggregation are major determining factors in the retention of incompletely assembled complexes in the ER.

We have also extended our previous studies on protein localization to the TGN to the dibasic endopeptidase, furin. The cytoplasmic domain of furin has been found to encode information specifying localization to the Golgi system. Together with our earlier studies on the TGN protein, TGN38, these findings strongly suggest the existence of a general mechanism for protein localization to the Golgi system that relies on the recognition of cytoplasmic signals. In order to identify genes involved in this mechanism, we have used a genetic screen for yeast mutants defective in the localization of the yeast homologue of furin, kex2p. This approach has yielded several mutant clones that are currently being further characterized genetically and biochemically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01608-03 CBMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Gene Regulation in Response to Enviromental Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Storz Staff Fellow CBMB, NICHD

Others: M. Toledano Courtesy Associate CBMB, NICHD

S. Altuvia Visiting Associate CBMB, NICHD

I. Kullik Special Volunteer CBMB, NICHD

E. Bruggemann IRTA CBMB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This group studies how organisms sense environmental signals and transduce the signal into changes in gene expression and cell morphology. The group has continued their characterization of the bacterial response to hydrogen peroxide. Two regulators are central to the response to hydrogen peroxide: OxyR, a protein activator that is switched on by oxidation, and OxyS, a novel 107 nucleotide RNA that acts as an activator and repressor of gene expression. The group has defined functional domains within the OxyR protein and elucidated the nature of OxyR-DNA recognition. Active domains within the OxyS RNA and a response element in an OxyS-regulated gene have also been identified. OxyS was found to act at the level of mRNA translation. Using *Arabidopsis thaliana* as a model system, the group has also initiated a large scale screen for mutants defective in their response to blue light.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01609-02 CBMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localization and Dynamics of Intracellular Organelles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Lippincott-Schwartz, Senior Staff Fellow, CBMB, NICHD

Others: N. Cole, Adjunct Scientist, CBMB, NICHD

A. Marotta, Pre-Irta, CBMB, NICHD

N. Sciaky, IRTA, CBMB, NICHD

COOPERATING UNITS *(if any)*

University of Texas Health Center (G. Bloom)
Laboratory of Neurobiology, NINDS, NIH (M. Terasaki)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

How membrane-bound organelles of eukaryotic cells maintain their identity and subcellular localization amidst an enormous input and outflow of membrane and protein is a central question in cell biology. Studies in this group have focused on this question and have sought to define the cellular and molecular mechanisms which underlie the organization and distribution of eukaryotic organelles. Particular attention has been paid to the Golgi complex which plays a fundamental role in the processing and sorting of protein moving through the secretory pathway. The Golgi complex in higher eukaryotes consists of stacks of flattened cisternae usually localized to the perinuclear region near the microtubular organizing center (MTOC). Recent studies have suggested this organization and positioning of the Golgi are controlled by dynamic processes. Tubulovesicular structures emerging from Golgi elements along microtubules, for example, enable adjacent Golgi stacks to communicate. In addition, reversible dispersal of Golgi elements occurs during microtubule disruption, mitosis and brefeldin A (BFA)-treatment. To further understand these processes and their relationship to the three-dimensional morphology and function of the Golgi we have taken four major approaches. 1) Imaging of the Golgi complex in living cells. Using the vital dye BODIPY-ceramide to label the Golgi complex in living cells we have performed time-lapse imaging to examine the dynamics of the Golgi complex in normal and BFA-treated cells. The important role of membrane tubulation in the normal dynamics and maintenance of Golgi structure within cells is under investigation. 2) Role of microtubules in Golgi localization and traffic. We have found that microtubules are important for both localizing the Golgi complex to the MTOC and for facilitating anterograde (ER-to-Golgi) and retrograde (Golgi-to-ER) membrane traffic into and out of this central region. Our characterization of Golgi dispersal induced by microtubule-disruption has suggested a mechanism involving membrane transport of Golgi components along retrograde and anterograde membrane pathways rather than diffusion or actin/myosin based transport. The role of microtubules in facilitating and directing membrane traffic to and from the Golgi, therefore, is likely to underlie the subcellular location of this organelle. 3) Role of microtubule motors in Golgi traffic. We have found that the microtubule motor protein, kinesin, associates with the anterograde and retrograde pathways leading to and from the Golgi complex. Conditions of microtubule disruption and low temperature treatments, which slow retrograde traffic, result in a large accumulation of kinesin on Golgi membranes. Factors that might regulate kinesin motor activity as this molecule cycles through anterograde and retrograde pathways are under investigation. 4) Role of Golgi positioning in late processing events of the secretory pathway. We have found that microtubule-dependent positioning of the Golgi complex to the MTOC is likely to facilitate the interaction of components moving through secretory and endocytic pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01610-01 CBMB

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intracellular Metal Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD

Others: A. Dancis, Expert, CBMB, NICHD
M. Diglio, EBON, CBMB, NICHD
D. Haile, Medical Staff Fellow, CBMB, NICHD
Y. Iwai, Visiting Fellow, CBMB, NICHD
E. Rabinovich, Guest Researcher, CBMB, NICHD
R. Stearman, Guest Researcher, CBMB, NICHD
D. Yuan, Clinical Associate/Medical Staff Fellow, CBMB, NICHD

COOPERATING UNITS *(if any)*

University of Utah, J. Kaplan

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.55	3.45	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the fundamental nutritional paradoxes that all cells must cope with is the requirement for metals that are both essential for cellular function yet extremely toxic. The toxicity of these metals, in particular iron and copper, is based upon their redox activity and their ability to directly mediate the oxidation of macro-molecules (metal catalyzed oxidation) or to indirectly damage cells via the generation of reduced oxygen intermediates. Iron poses an addition problem for cells because, in the presence of oxygen, it is found in the insoluble ferric form. This project is aimed at understanding the molecular mechanisms responsible for the uptake, sensing, regulation, intracellular distribution, and utilization of these toxic but essential metals. This work has been approached using the simple eukaryote, *Saccharomyces cerevisiae*, which allows us to apply powerful molecular and genetic tools to the identification and characterization of essential components of metal metabolism. Through this approach, we have identified a series of genes whose function define the interlocking mechanisms by which both iron and copper are acquired from the environment by eukaryotic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01802-04 CSB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Data Coordinating Center for the NICHD Study of Early Child Care

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.K. Knoke	Computer Specialist	CSB, DESPR, NICHD
Other:	Mark Appelbaum	Methodologist/IPA	CSB, DESPR, NICHD

COOPERATING UNITS (if any)

CRMC, NICHD/NIH

LAB/BRANCH: Computer Sciences Branch, DESPR

SECTION: Data Coordinating Center

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	1.1	3.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is a 10-site cooperative agreement to examine the effects of non-maternal care, especially during the first year of life, on the social and intellectual development of children in a ecological framework which takes into account the complex interactions of non-maternal care experiences with home and family conditions, parenting practices, and child characteristics. Over 1200 families were enrolled in the study during 1991. These families will participate in the study for 36 months.

The Data Coordinating Center (DCC) is responsible for data management, protocol monitoring and analysis. It serve as the data coordination center and is the focal point for receipt of collected data and data processing.

The DCC is currently designing forms and writing and editing chapters of the Operations Manuals for 36-month data collection procedures. Concurrently through 18-month, 24-month and 30-month data are being received from the sites, entered to computer files, edited and summarized for monitoring purposes. Routine edit reports are being distributed to the sites regularly. Project monitoring reports are distributed to the Steering Committee quarterly.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-13 DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth, Puberty, Their Disorders: Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Gordon B. Cutler, Jr., M.D. Head SDE, DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
9.5	8.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Since somatic growth is a major determinant of the timing of pubertal onset, a further objective is to clarify the mechanisms of normal growth and of growth failure. Principle areas of laboratory investigation include the mechanisms of normal growth and puberty. Principle areas of clinical investigation include the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone-releasing hormone (LHRH), the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor, the evaluation of new approaches to the diagnosis of growth hormone deficiency and to the differential diagnosis of delayed puberty, the treatment with synthetic parathyroid hormone of children with hypoparathyroidism, the treatment with growth hormone of children w' Turner syndrome and with non-growth hormone-deficient short stature, and the treatment with insulin-like growth factor-1 of children with Laron short stature and of children with idiopathic short stature. The principal areas of laboratory investigation include the structure and function of the regulatory sequences of the human gonadotropin-releasing hormone (GnRH) gene, the structure of the human GnRH gene in families with GnRH-dependent precocious puberty or with idiopathic hypogonadotropic hypogonadism, molecular analysis of the gene for the luteinizing hormone receptor in familial male precocious puberty, and hormonal regulation of epiphyseal transforming growth factor-β, fibroblast growth factor, and platelet derived growth factor. To examine the effects of these growth factors in vivo we are studying rabbits bearing small needles implanted into the proximal tibial epiphyses and connected to osmotic minipumps.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00615-13 DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocrine-Immune Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G.P. Chrousos	Head	SPE, DEB, NICHD
Others:	G. Mastorakos	Visiting Fellow	SPE, DEB, NICHD
	M. Reincke	Adjunct Scientist	SPE, DEB, NICHD
	N. Vamvakopoulos	Expert	SPE, DEB, NICHD
	E. Webster	PRAT Fellow	SPE, DEB, NICHD
	K. Zachman	Bio Lab Technician	SPE, DEB, NICHD

COOPERATING UNITS *(if any)*

P.W. Gold, E. Sternberg, Clinical Neuroendocrinology Branch, NIMH; R. Wilder, Arthritis and Rheumatism Branch, NIAMS; Gabriel Panayi, University of London, UK.

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Pediatric Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. Antagonists for the glucocorticoids or the progestins with potential clinical usefulness have been discovered recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids. We have tested a prototype glucocorticoid-progestin antagonist (RU 486) developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals. Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that antiglucocorticoids could be used for challenging the hypothalamic-pituitary-adrenal axis, when clinical testing is required in patients with disorders of this axis. Antiglucocorticoid therapy of patients with severe Cushing's syndrome due to ectopic ACTH secretion or adrenocortical tumors causes remission of the clinical manifestations of hypercortisolism. RU 486 potentiated the inflammatory/immune response to a standard stimulus in intact animals, suggesting that glucocorticoids exert anti-inflammatory/immunosuppressive effects at physiological levels. We recently demonstrated that corticotropin releasing hormone (CRH) is produced locally at the site of inflammation and has profound pro-inflammatory effects at an autocrine/paracrine level. We have called this "immune" CRH. Glucocorticoids suppress, and RU 486 markedly augments local secretion of immune CRH at an inflammatory site. Immune CRH was found in the ovary and endometrium where it may participate in the inflammatory phenomena of ovulation, luteolysis, and menstruation. RU 486 allowed the identification of a central nervous system defect in rats prone to arthritis. In these animals the glucocorticoid response to stress-mediators is inadequate to restrain the immune system following an insult. The actual defect is global and located at the level of the hypothalamic CRH neuron, which responds poorly to all its known stimulants, including several cytokines, as well as serotonin, acetylcholine and norepinephrine. This pathophysiologic mechanism is novel and of relevance to human arthritis and autoimmune disease. Patients with rheumatoid arthritis have defective pituitary-adrenal axis responses to inflammatory stimuli and produce excessive amounts of immune CRH in their inflamed joints. The human CRH gene contains estrogen-responsive elements in its promoter region providing an explanation for the sexual dimorphism of the higher incidence of autoimmune/inflammatory disease in women than men.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00616-13 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure, Function, and Physiology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	B.C. Nisula	Head	SME, DEB, NICHD
Others:	D. Blithe	Expert	SME, DEB, NICHD
	B. Olson	Senior Staff Fellow	SME, DEB, NICHD
	M. Flack	Clinical Associate	SME, DEB, NICHD
	D. Rubino	Clinical Associate	SME, DEB, NICHD
	J. Froehlich	Visiting Fellow	SME, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general goal of this project is to understand the structure, function and physiology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FHS), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances in the current period include the following: First, several βFSH domains involved in FSH action were identified by using a novel technique for assessing the signal transducing activity of FSH mutants produced by site-directed mutagenesis. The data show that both of the βFSH glycosylation sites have a role in FSH signal transduction; at the first site oligosaccharide is the critical moiety for efficient signalling, while at the second site the amino acid sequence is crucial for hormone activity, independent of the oligosaccharide. Second, the ability of human TSH to interact with and activate the human FSH receptor was established. This provides a novel pathophysiologic mechanism for the syndrome of precocious puberty and juvenile hypothyroidism. Third, for the first time direct demonstration of the ability of hCG to stimulate thyroid hormone secretion from human thyrocytes has been accomplished, thereby resolving a longstanding controversy. Fourth, the ability of GnRH antagonist to ameliorate murine lupus was shown, setting on the horizon a possible role for this agent in the treatment of human autoimmune diseases with this type of agent. Fifth, the presence of IGF-I and IGF-II receptors on GnRH-producing neurons has been demonstrated, suggesting a possible role of growth factors in the regulation of gonadotropin secretion. Future directions of the project will emphasize study of the structure-function characteristics of human FSH and the human FSH receptor through site-directed mutagenesis of their respective genes, and investigation of the signal transduction mechanisms in human thyrocytes in vitro with analogs of hCG.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00618-12 DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.P. Chrousos	Head	SPE, DEB, NICHD
Others:	K. Arai	Visiting Fellow	SPE, DEB, NICHD
	G. Cizza	Adjunct Scientist	SPE, DEB, NICHD
	L. Dorn	Adjunct Scientist	SPE, DEB, NICHD
	A. Magiakou	Adjunct Scientist	SPE, DEB, NICHD
	(continued on attached list)		

COOPERATING UNITS *(if any)*

See attached list.

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Pediatric Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	4.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic-pituitary-adrenal axis. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas: 1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's states (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, melancholic depression, atypical depression, seasonal depression, the premenstrual tension syndrome, anorexia nervosa, the chronic fatigue syndrome late-onset congenital adrenal hyperplasia, diabetes mellitus and rheumatoid arthritis. 2) The regulation of the axis by opioids, vasopressin, oxytocin, glucocorticoids, eicosanoids, platelet activating factor (PAF), tumor necrosis factor - alpha, cholecystokinin (CCK), neuropeptide Y, benzodiazepine agonists and antagonists and thyroid hormones has been studied in vivo and/or in vitro. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has also been examined in vitro and in vivo. Third trimester pregnant women and athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents. The hCRH gene 5' regulatory region has been cloned and sequenced and its regulation is being studied. It has 2 active promoters and responds positively to estrogens, providing a potential explantation for the sexual dimorphism of psychiatric diseases characterized by abberations in CRH secretion. 3) Role and actions of glucocorticoids: Surgical stress is associated with continuous and pulsatile secretion of ACTH. Glucocorticoid resistance is an autosomal recessive or dominant disease associated with abnormalities of the glucocorticoid receptor type II. We have elucidated the molecular pathophysiology of this syndrome by defining mutations and/or deletion of the glucocorticoid receptor gene leading to abnormal or decreased receptors in the tissues of patients. The glucocorticoid receptor type I (mineralocorticoid) is studied in several kindreds with mineralocorticoid resistance. The interaction of the glucocorticoid receptor with the heat-shock proteins is studied. Hereditary ACTH resistance is an autosomal recessive disorder characterized by pituitary glucocorticoid deficiency. We have elucidated the molecular pathophysiology of this syndrome by defining abnormalities of the ACTH receptor gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-11-DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and Treatment of Growth Disorders.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. Cassorla	Head	UGP, DEB, NICHD
Others:	H. Domené	Visiting Associate	UGP, DEB, NICHD
	Y. Yu	Visiting Associate	UGP, DEB, NICHD

COOPERATING UNITS *(if any)*

D. Le Roith, Diabetes Branch, NIDDK

S. Rose, Department of Pediatrics, University of Tennessee, Memphis

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Growth Physiology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5		0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include improving the accuracy of the methods employed to diagnose growth hormone deficiency. We are also studying the effects of growth hormone and sex steroid administration on linear growth in patients with delayed puberty. In addition, we are studying the mechanism of catch up growth in a primate model. We are also studying the hormonal regulation of transcriptional expression of the growth hormone receptor gene in both rabbits and rats. In addition, we are examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to epiphyseal fusion. Finally, we are studying the effects of growth hormone-releasing factor and growth hormone releasing-peptide on linear growth in growth hormone-deficient children by using different treatment regimens in order to optimize growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00623-10 DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adrenal Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Gordon B. Cutler, Jr. Head SDE, DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

J. Doppman, Radiology, CC; E. Oldfield, Surgical Neurology Branch, NINDS; J. Booth, Dept. Internal Medicine, McMaster University Medical Center; S. Radovick, F. Wondisford, Dept. Pediatrics and Internal Medicine, Harvard Medical School; D.L. Loriaux, Oregon Health Sciences University

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.9	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.

Children with congenital adrenal hyperplasia are being enrolled into one of four projects. The first tests the effect of different dose-schedules of hydrocortisone. The second examines the hypothesis that supplemental salt intake will reduce the day-to-day variability, and improve the overall control of androgen secretion. The third examines the adrenocorticotropin-suppressing activity of carbenoxolone, an inhibitor of 11-beta-hydroxysteroid dehydrogenase. The fourth employs a newly available antiandrogen and an inhibitor of androgen-to-estrogen conversion to block the action of excess androgen levels. Additionally, the potential to cure the 21-hydroxylase deficiency form of congenital adrenal hyperplasia by adrenal transplantation or gene therapy is being explored in a newly recognized animal model of this disorder.

Patients with Cushing syndrome are being studied by several new diagnostic methods to determine the relative diagnostic efficiency of these new methods compared to the old. Oncogene expression in corticotropinomas is also under study in an attempt to elucidate the molecular basis of Cushing disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00626-05-DEB

PERIOD COVERED

October 1, 1992 to September 30, 1993.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Progesterone Action in Reproduction.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. K. Nieman	Senior Investigator	URM, DEB, NICHD
Others:	D. Johri	Visiting Fellow	URM, DEB, NICHD
	F. Sharara	Visiting Associate	URM, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Progesterone is essential for the normal morphologic development of the luteal phase endometrium, for implantation of a blastocyst and for maintenance of pregnancy. Surprisingly, given its critical role in reproduction, little is known about the mediator(s) of progesterone action. We are using a recently described progestin-dependent glycoprotein, placental protein 14 (PP14) and the antiprogestin RU 486 to probe progesterone action. We are investigating the relationship between endogenous progesterone production, serum PP14 concentrations and endometrial maturation in women with normal cycles. Up to 28% of normally cycling women had a delay in endometrial maturation of a biopsy obtained 7 - 9 days after the LH surge. Contrary to previous studies from other groups, the integrated and peak levels of PP 14 and progesterone did not correlate with the degree of endometrial maturation. Only two of 14 women having abnormal endometrial development had progesterone values below the range seen in women with normal endometrial development. These studies suggest that measurement of serum PP14 and progesterone concentrations may not be useful predictors of fecundity in normally cycling women. Further studies in infertile women and those with multiple miscarriages may give better insight into the cause of these disorders, however, since it is possible that the progesterone-mediated endometrial effects may be abnormal in these subgroups.

We have found that IGF-1 and the proto-oncogene c-myc appear to mediate the effects of estrogen exposure and withdrawal on the processes of uterine proliferation and apoptotic regression in the mouse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00627-04 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glycoprotein Hormones: Oligosaccharide Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	D.L. Blithe	Expert	MES, DEB, NCIHD
Others:	B. Paindaveine	Visiting Fellow	MES, DEB, NICHD
	C. Lyons	Bio Lab Technician	MES, DEB, NICHD

COOPERATING UNITS *(if any)*

R. Isles, St. Barthomomew's Hospital Medical College, West Smithfield, London, U.K.

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	2.6	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of the project is to increase our understanding of the structure-function relationships and glycobiology of the oligosaccharide moieties on glycoprotein hormones. The molecules currently under investigation are, human chorionic gonadotropin (hCG), gonadotropin free α subunits associated with pregnancy or malignancy, and pituitary free α subunits secreted throughout the normal menstrual cycle. We have shown that the free α subunit purified from pregnancy urine stimulates secretion of prolactin from primary cultures of human decidual cells in a dose-dependent manner. These findings indicate that free α may be a glycoprotein hormone with a function that is independent of hCG.

Carbohydrate modifications, resulting in a variety of branched oligosaccharide structures, occur on all glycoproteins prior to secretion. These modifications can affect virtually every aspect of the molecule's behavior, including receptor binding and signal transduction, yet the underlying regulatory mechanisms remain elusive. We have investigated functional aspects of the oligosaccharide moieties on free α, demonstrating that specific types of carbohydrate modifications on free α can either prevent or facilitate combination with hCG-β to form intact hormone.

We have found that the free α subunits that are located in fetal compartments in high concentrations during early pregnancy exhibit physical properties that are similar to those of the free α subunit isolated from pregnancy urine. It is likely that oligosaccharide moieties on this early pregnancy free α function to maintain the molecule in an uncombined form.

We have identified differences in the oligosaccharide moieties of free α and hCG as a function of gestational development; in particular, the glycosylation of free α changes dramatically as a function of gestational age. Molecules produced in late pregnancy are more highly branched and are much more extensively fucosylated than those of early pregnancy. We plan to examine how the differences observed in glycosylation affect the bioactivity of these molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00628-04 DEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Paracrine and Autocrine Roles of Insulin-like Growth Factors (IGFs).

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C. A. Bondy	Senior Investigator	EGFU, DEB, NICHD
Others:	J. Zhou	Visiting Fellow	EGFU, DEB, NICHD
	E. Chin	Senior Staff Fellow	EGFU, DEB, NICHD
	R. Reinhardt	Medical Staff Fellow	EGFU, DEB, NICHD
	M. Bach	Senior Staff Fellow	EGFU, DEB, NICHD
	X. Wu	Bio Lab Technician	EGFU, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Endocrine Growth Factor Unit

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the autocrine/paracrine functions of insulin-like growth factors I and II (IGF-I and -II) in the central nervous system, reproductive system, and kidney in both rodents and humans. Our major experimental methods include in situ hybridization, immunocytochemistry and ligand-binding autoradiography; these techniques are employed to identify the cellular sites of synthesis of the growth factors. In addition, we have used these same methods to determine the cellular localization of the receptors which mediate IGF actions and to map the distribution of the six different high-affinity IGF binding proteins which are known to modulate the interaction of IGF-I and -II with their receptors. Our studies have focused on normal embryonic development as well as a number of different physiological and pathophysiological situations in which IGFs seem to be critically important.

Important results of these studies during the past year include: 1) Evidence for the expression of hybrid insulin: IGF receptors which have predominantly metabolic effects but demonstrate higher affinity for IGF-I than insulin. This hybrid receptor is preferentially expressed in brain and kidney. 2) A new member of the IGF/insulin receptor family, termed the insulin receptor-related receptor (IRR) has been identified, and we have shown that it is selectively colocalized with the high-affinity nerve growth factor (NGF) receptor. Data suggests that this new receptor is physically or functionally linked with the NGF receptor and together they are involved in determining the survival of sensory and sympathetic neurons. 3) IGF-I (in the rodent) and IGF-II (in the human) appear to be critical determinants of ovarian follicular maturation and ovulation, and expression of one of the specific (inhibitory) IGF binding proteins by a follicle appears to predict follicular atresia and oocyte demise. Furthermore, there is intense IGF-I receptor expression in human oocytes and spermatocytes. These findings have very significant clinical implications in terms of the potential utilization of IGFs or IGF or analogs to selectively regulate cerebral and renal metabolism and both male and female fertility.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00629-03 DEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polyol Pathways in Health and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	C.A. Bondy	Senior Investigator	EGFU, DEB, NICHD
Others:	Z. Jian	Visiting Fellow	EGFU, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Endocrine Growth Factors Unit

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Polyhydric alcohols, or polyols, are osmotically active organic molecules whose physiologic and pathophysiologic significance has only very recently come under investigation. These molecules are produced by the reduction of common sugars via the catalyic action of aldose reductase. It appears that the overproduction and accumulation of polyols such as sorbitol, the product of glucose reduction, in certain tissues, may be responsible for some of the dire consequences of chronic hyperglycemia, as in diabetes mellitus or hereditary galactosemia.

discontinued

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PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00631-03 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G. Aguilera	Head	SEP, DEB, NICHD
Others:	A. Kiss	Visiting Associate	SEP, DEB, NICHD
	Q. Pham	Student Trainee	SEP, DEB, NICHD

COOPERATING UNITS *(if any)*

S. Lightman, Department of Medicine, University of Bristol, U.K.
R.L. Hauger, Department of Psychiatry, University of California, San Diego

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation has focused on the mechanism of regulation of the hypothalamic and pituitary function during stress. These studies have defined distinct types of responses of the hypothalamic-pituitary-adrenal axis during adaptation in different chronic stress paradigms: a) desensitization of the ACTH to the primary stress, but hyperresponsiveness to a novel stimulus, b) sustained ACTH response to the primary stimulus and hyperresponsivenes to a novel stimulus, and c) minor ACTH responses to the primary stimulus and reduced sensitivity to a novel stress. To determine the mechanism of this differential responses, the hypothalamic expression and secretion of the major regulators of ACTH secretion, corticotropin releasing hormone (CRH) and vasopressin (VP), were studied in the various stress paradigms. A major finding was that activation of the HPA axis during chronic stress is accompanied by activation of the parvicellular vasopressinergic system, which provides VP to the pituitary portal blood. On the other hand, increasing systemic VP levels by osmotic stimulation failed to potentiate the effects of CRH on ACTH secretion and CRH receptor regulation. At the pituitary level, increased ACTH responses were associated to increases in POMC mRNA levels, but no relationship was found between CRH receptor levels and pituitary responsiveness. Previous studies demonstrated that alpha-1 adrenergic receptors are important mediators of stress-induced release from the median eminence. Extension f these studies, using electron microscopy immunocytochemical techniques, showed that α1-adrenergic agonists stimulate CRH release from the subpopulation of VP-containing CRH neurons. Additional studies provided two types of evidence indicating that long term, but not acute central α1-adrenergic receptor activation increases CRH mRNA in the hypothalamus. First, repeated injection of the α1-receptor agonist, methoxamine, every 6 hr for 24 hr, but not 4 hr after a single injection, resulted in significant increases in CRH mRNA in the PVN, and second, central α1-adrenergic blockade prevented the increases in CRH mRNA observed after 24 hr stress, but not those after 4 hr stress. Studies are in progress to determine the precise role of the multiple neurotransmitters implicated in hypothalamic regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00632-04 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Actions of the Renin-Angiotensin-System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G. Aguilera	Head	SEP, DEB, NICHD
Others:	P. Feuillan	Medical Officer	SEP, DEB, NICHD
	B. Akbasak	Visiting Associate	SEP, DEB, NICHD

COOPERATING UNITS *(if any)*

P. Ray, Laboratory of Developmental Biology, NIDR, NIH

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study physiological and pathological aspects of the renin-angiotensin system, with emphasis on its role on circulatory homeostasis and development. Studies in neonatal rats showing decreased aldosterone responses to angiotensin II (AII) associated to the presence of abundant adrenal AT2 receptors suggested developmental changes in the regulation of the adrenal glomerulosa function. In collagenase dispersed adrenal glomerulosa cells from 7-day old rats, blockade of AT2 receptors had no effect on the stimulation of aldosterone production by AII, indicating that AT2 receptors are not involved in the steroidogenic actions of AII. An important finding was that in contrast to adult rats, plasma aldosterone levels stimulated by AII and ACTH and the last step of the aldosterone biosynthetic pathway, aldosterone synthetase, were markedly inhibited by administration of dexamethasone 18 hr earlier. Preincubation of isolated adrenal cells with dexamethasone for 2 hr had no effect on basal or stimulated aldosterone production. The inhibitory effect in vivo, was not prevented by ACTH suggesting that in neonatal rats aldosterone secretion is under the influence of a glucocorticoid sensitive factor different from ACTH. Continuation of studies on the effects of AII in human kidney mesangial cells revealed that incubation of confluent cell cultures with AII caused significant increases in fibronectin mRNA level and fibronectin synthesis. The stimulatory effect of AII in the expression of extracellular matrix pro ins suggest the involvement of AII in the pathogenesis of glomerular disease. The role of AII receptor changes in the altered vascular responsiveness to AII in portal hypertension was studied in a surgical model of portal hypertension in the rabbit. AII receptor content was significantly decreased in vascular smooth muscle membranes from mesenteric artery and portal vein of portal hypertensive rabbits. This may explain the blunted pressor responses to AII observed in portal hypertension. The topographic distribution of AII receptor mRNA was studied in the rat brain using cRNA probes obtained by PCR using primers based on the sequence of AT1 receptor and cDNA from rat brain. AT1 receptor mRNA was located in ares known to contain AT1 receptor binding. In addition, expression of AT1 receptor mRNA in areas in which binding has not been shown, such as the hippocampus and arcuate nucleus indicate that the presence of low levels of receptors may explain the reported biological effects of AII in these areas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00633-03 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ovarian folliculogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	L.M. Nelson	Staff Clinical Specialist	SGR, DEB, NICHD
Others:	Z.B. Tong	Visiting Fellow	SGR, DEB, NICHD
	J. Anasti	Medical Staff Fellow	SGR, DEB, NICHD
	S. Nair	NRSA Fellow	SGR, DEB, NICHD
	J. Jacobson	Medical Staff Fellow	SGR, DEB, NICHD
	R. Maity	Visiting Fellow	SGR, DEB, NICHD

COOPERATING UNITS *(if any)*

J. Dean, Mammalian Developmental Biology Section, Laboratory of Cellular and Developmental Biology, NIDDK

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Gynecologic Research Section

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to understand the physiology and pathophysiology of ovarian follicle development and function, and to thereby develop clinical diagnostic and therapeutic applications for women with ovulatory disorders. During this period we have focused on premature ovarian failure, a condition which prematurely terminates fertility in 1% of women. We have particular interest in autoimmune-mediated ovarian failure and seek to improve our understanding of this condition through clinical research and through basic immunologic research employing a mouse model of autoimmune ovarian failure (induced by neonatal thymectomy). Previously we demonstrated that during a 4 month observation period with serial serum estradiol measurements, we can detect ovarian follicle function in approximately 50% of patients with premature ovarian failure. Furthermore, almost 20% of these patients ovulated during this observation. During this period we completed patient participation in a controlled evaluation of danazol therapy as a means to induce remission in patients with ovarian failure. We are now collating and analyzing the data generated in this study. Also during this period, we initiated a clinical protocol to evaluate alternate-day prednisone therapy of premature ovarian failure in patients with biopsy-proven lymphocytic oophoritis. We are also determining the significance of antibodies directed against the zona p· ıcida and against the FSH receptor. During the current year we have continued our work in a mouse model of autoimmune lymphocytic oophoritis. We have defined the time course and histologic location of the ovarian lymphocytic infiltrate by using immunohistochemistry and a monoclonal antibody directed against a surface protein specific to mouse leukocytes (CD45). We are using this technique to evaluate the role of gonadotropins in the initiation of this disease. We are treating mice with gonadotropins to see if we can accelerate the disease and we are suppressing gonadotropins with a gonadotropin releasing hormone antagonist to see if we can delay or prevent the disease. We are also evaluating the role played by the H-2 complex in this disease by studying the effect neonatal thymectomy has on congenic strains of mice. We are also investigating other methods to induce lymphocytic oophoritis in mice by altering the T cell repertoire in the neonatal period using pharmacologic agents. In the coming years we also plan to evaluate other treatments for premature ovarian failure by controlled prospective clinical trials and to assess the pathophysiologic role of immunoglobulins and T lymphocytes in autoimmune premature ovarian failure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00634-02 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Effects of insulin-like growth factor I in normal and diabetic adocescents.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	C.A. Bondy	Senior Investigator	EGFU, DEB, NICHD
Others:	Mark Bach	Senior Staff Fellow	EGFU, DEB, NICHD
	Edward Chin	Senior Staff Fellow	EGFU, DEB, NICHD
	Rick Reinhardt	Medical Staff Fellow	EGFU, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Endocrine Growth Factors Unit

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many insulin-dependent diabetic (IDDM) adolescents have elevated growth hormone (GH) and low insulin-like growth factor-I (IGF-1) levels. These hormonal abnormalities contribute to insulin-resistance and further exacerbate already compromised mechanisms of fuel homeostasis. This study compared baseline IGF and IGF binding protein (IGFBP) levels and responses to treatment with recombinant IGF-I in normal and diabetic male adolescents. Subjects were studied before and after daily 10 hour subcutaneous infusions of saline or IGF-I (20 μg/kg/hour). Baseline IGF-I levels were almost 50% lower and IGF-II levels were significantly higher in diabetics compared to controls. IGF-I infusion produced an increase of 3-4 fold in serum IGF-I levels and a profound reduction in IGF-II levels. IGFBP1 levels increased dramatically in diabetics and modestly in normals during two days of IGF-I infusion but IGFBP3 levels were not affected. Spontaneous overnight and arginine-stimulated GH secretion were suppressed by approximately 50% in both groups following IGF-I infusion. Insulin requirements were substantially reduced in diabetics receiving IGF-I and insulin secretion was suppressed in normals, with no evidence of a change in insulin half-life. No hypoglycemia or other adverse effect occurred during IGF-I treatment. These findings suggest that treatment of adolescent diabetics with IGF-I in addition to insulin may achieve better metabolic and anabolic balance than treatment with insulin alone. Long-term studies are now required to determine if these effects are sustained and to evaluate the effects of normalizing IGF-I and GH levels on growth and on diabetic complications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00635-01-DEB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of GnRH Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. K. Nieman	Senior Investigator	URM, DEB, NICHD
Others:	B. Olson	Senior Staff Fellow	URM, DEB, NICHD
	D. Scott	Medical Staff Fellow	URM, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulsatile secretion of GnRH by hypothalamic neurons results in gonadotropin secretion and release and hence plays a critical role in the regulation of normal reproductive function and cyclicity in women. The central mechanisms that regulate neuronal function are not understood. Chronic nutritional deprivation in people and primates causes hypothalamic dysfunction and hypogonadism, but is not a clinically acceptable model. Recent work indicates that acute food deprivation in men and male monkeys decreases gonadotropin pulse frequency and testosterone levels. We hypothesize that acute fasting may inhibit the female reproductive axis in a similar way, and may serve as a tool to study the regulation of the GnRH pulse generator. Short-term fasting, common among women, might thus inhibit the reproductive axis and diminish fertility.

To study the effects of nutritional deprivation on GnRH neuronal function, we have fasted women completely for three days during the follicular phase of the menstrual cycle. Preliminary data show that this reversibly blunts follicular growth but does not decrease gonadotropin pulsatility, suggesting that even a 3 day fast can affect follicular development, primarily at the ovary, and that species-specific and sex-specific differences exist in the activity of the GnRH pulse generator during a fast. We have also used the GT1-7 neuronal cell line to study the regulation of GnRH secretion in vitro. This line is derived from a transgenic mouse transfected with the sequences for the rat GnRH promoter and the SV40 T antigen. We have shown that neuronal survival and GnRH secretion by these cells is affected by the glucose and IGF-1 concentrations in the media, so that glucose-free media and supra-physiologic concentrations of IGF-1 increase cell death, while a physiologic concentration of IGF-1 protects the neurons from hypoglycemic damage. GnRH secretion is inhibited by IGF-1, 1 -100 ng/mL. These data suggest that the hypogonadism of starvation may be modulated directly at the level of the GnRH neuron by the availability of nutrients and growth factors.

TERMINATED

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD-00636-01 DEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocrinology of Reproduction in Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.H. Segars	Staff Clinical Specialist	SGR, DEB, NICHD
Others:	S. Nunez	Clinical Associate	SGR, DEB, NICHD
	D. Arbit	Special Volunteer	SGR, DEB, NICHD
	G. Leong	Visiting Associate	SGR, DEB, NICHD

COOPERATING UNITS *(if any)*

K. Ozato, SMGI, Laboratory of Molecular Growth Regulation, NICHD

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Molecular Mechanisms of Reproduction

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Overall objective of this project is to understand the endocrinology of hormonal responses in women (e.g., the estrogen response) in order to facilitate the development of clinical therapies directed toward the problems of contraception, pregnancy loss, and fertility. Despite the fact that the estrogen response is central to reproduction, little is known about the ability of other nuclear hormone receptors to influence estrogen receptor function. Recent work has therefore focused on factors modulating the estrogen response in estrogen responsive tissues. Advances in the current period include: 1) Definition of the role of the retinoid X receptor (RXRß) in the estrogen response. Cotransfection studies have shown that overexpression of RXR leads to inhibition of estrogen-dependent gene activation. Further, this inhibition is specific for gene activation caused by the estrogen receptor, since inhibition was only observed in estrogen receptor negative cells following introduction of the estrogen receptor. The inhibition was specific for the estrogen response since glucocorticoid-mediated gene activation was not impaired; 2) Demonstration that the RXR receptor was present in estrogen responsive cells in conjunction with an auxiliary factor that bound to estrogen responsive DNA elements; and 3) Demonstration that RXR and thyroid receptor (TR) heterodimers also bound to estrogen responsive DNA sequences and impaired gene activation by the estrogen receptor. We plan to examine the role of other receptors (known to interact with RXR) to determine whether those receptors may play a role in the modulation of estrogen responsive genes as well. In addition, we plan to study the endogenous auxiliary factor which contributes to binding of estrogen responsive DNA sequences by RXR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00329-11 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.W. Berendes	Director	DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD

COOPERATING UNITS (if any)
Greater Washington Research Center, Washington, DC (J. Maxwell); Better Babies Project, Washington, DC (D. Coates)

LAB/BRANCH
Office of the Director/Epidemiology Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a community based intervention in a geographically defined area of Washington, D.C., which comprises nine census tracts. The major objective of this community based intervention was the reduction of low birth weight in the target community. This was a multi-risk factor intervention targeted upon pregnant women which included an extensive effort to identify and recruit pregnant women who lived in the target area early in pregnancy, to link them to prenatal care and for those in prenatal care, to enhance compliance, to provide social support and facilitate access to entitlements for which these women may be eligible, such as Medicaid, WIC, Commodity Surplus Food, AFDC, as well as others. In addition, interventions were offered for adverse health behaviors like smoking, alcohol and substance abuse. The study recruited pregnant women over a period of three years from September 1986 through September 1989. The last women recruited in this project delivered in the Spring of 1990.

A detailed analysis of the impact of the intervention on the target community has been completed and a manuscript written and submitted for publication.

This project is completed.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00343-10 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Westernization on Infant Feeding Patterns Among the Negev Bedouins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.W. Berendes Director DESPR, NICHD

COOPERATING UNITS (if any)

Cancer Prevention Branch, NCI, NIH (M.R. Forman); Biometry Branch, DCPC, NCI (B. Graubard); Ben Gurion University of the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH

Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.05	.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a study of infant feeding practices among Arab Bedouins residing in the Negev, Israel. The objectives are: infant feeding practices at birth and changes during the first year of life and relationships to physical growth and to gastrointestinal and respiratory morbidity during the first 18 months of age.

The data base includes information on 5,000 mother-infant pairs, and represents the total population of Bedouin births during the years 1981 and 1982. Two subsamples have been identified for follow up; one at birth and followed for a period of 5-8 months. Another sample of children was identified at 6 months of age and followed prospectively to 18 months of age.

Two additional manuscripts have been published from this da' set during 1992, one dealing with the factors influencing milk insufficiency and its long term health effects and the other of the accuracy of maternal recall of infant feeding events.

This study is completed

004

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00878-02 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biological Effects of Delayed Childbearing

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Cnattingius	Visiting Scientist	DESPR, NICHD
Others:	H. Berendes	Director	DESPR, NICHD
	L. Isotalo	Volunteer	DESPR, NICHD
	M. Forman	Epidemiologist	CPSB, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There has been a marked increase in first births among women age 30 or more in the U.S. and the numbers have increased from 1975 to 1986 from 69,000 to 230,000 per year. There is a controversy as to whether there is an increased risk of adverse birth outcome among delayed childbearers. There is also a question whether the reported increase risk in adverse birth outcome is due to confounding by other factors.

This research is using a population based Swedish data set of more than 500,000 births from 1983 to 1987, which in addition to demographic characteristics includes information on medical and pregnancy complications, history of infertility, reproductive history, as well as smoking. This is a unique data set and we are not aware of any comparable population based information in the United States.

Three manuscripts have been published from this study.

This project is completed.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01701-05 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of the Impact of a Model Prenatal and Followup Program in Baltimore

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. W. Berendes	Director	OD, DESPR, NICHD
Other:	A. Herman	Visiting Scientist	EB, DESPR, NICHD
	B. Wingrove	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Department of Health, Baltimore City (Tom Coyle)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a community based multi-risk factor intervention in a geographically defined area of Baltimore designed to improve birth outcome, especially to reduce the rate of low birth weight in the target population. The initiative for this project has come from the Baltimore City Health Department. The Baltimore City Health Department, in turn, has requested technical assistance by the staff of DESPR in the design of the intervention and the evaluation of its impact upon the target population. This is a quasi-experimental design which will compare outcome measures which are available from vital records information for birth in the target area during the period of the project with outcome measures derived from other areas of Baltimore which are similar in population characteristics. In addition there will be a process evaluation which will address smoking, drinking and drug abuse as well as compliance with prenatal care and also knowledge and attitudes about appropriate health behaviors during pregnancy.

Patients have been enrolled in this project since December 1, 1990 and approximately 500 have been enrolled thus far.

This project has become the starting point and nucleus for the Healthy Start Initiative in Baltimore which is funding by HRSA. As a result of this development, this project has been terminated and the analysis of the data are in progress.

011

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01702-04 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Infant Feeding Practices Among North African Jews

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes — Director — DESPR, NICHD

COOPERATING UNITS *(if any)*
Cancer Prevention Branch, DCPC, NCI, NIH (M.R. Forman); Biometry Branch, DCPC, NCI, NIH (B. Graubard); Ben Gurion University of the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: .05	PROFESSIONAL: .05	OTHER: .0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a study of infant feeding practices among North African Jews who immigrated to Israel during the last decade. The objectives are the evaluation of infant feeding practices and changes during the first year of life in relation to physical growth and morbidity during the first three years of life. In addition it is intended to relate maternal nutritional status during pregnancy to choice and practice of infant feeding during the first few months of life.

The information obtained covers about 1,000 mother-infant pairs. The follow up of these children has been for three years.

The data collection for this project was supported in part by an interagency agreement between the Centers for Disease Control and the WHO.

This project is completed.

013

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01703-04 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Pregnancy Outcome, Maternal Death and Child Health in Pakistan

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. W. Berendes	Director	DESPR, NICHD
Other:	Fariyal Fikree	Visiting Fellow	DESPR, NICHD

COOPERATING UNITS (if any)
Department of Community Health, Aga Khan University (Drs. John Bryant, Fariyal Fikree, Farid Midhet, and Mehtab Karim), Special Program for Research, Reproduction and Family Planning of the WHO (Dr. Jose Villar)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has three components.

The Maternal and Infant Mortality Survey (MIMS) will provide estimates of maternal and infant mortality rates from population based samples in Karachi and the four provinces of Pakistan. In addition, the Survey will provide information on causes of maternal and infant deaths and demographic and other determinants of maternal and infant mortality.

The Pregnancy Outcome Study (POS) is a cross sectional and prospective study of pregnant women from selected katchi abadies to identify determinants of poor birth outcome in this population.

The Child Survival Study (CSS) is a follow up study of live born children from POS through two years of age to assess mortality, morbidity and physical and developmental measures.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01704-03 ESPR

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stunting Among Bedouin Arab Children in the Negev, Israel

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes — Director — DESPR, NICHD

COOPERATING UNITS *(if any)*
Cancer Prevention Studies Branch, DCPC, NCI, NIH (M.R. Forman); Ben Gurion University of the Negev, Beer Sheva, Israel (G. Hundt)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	.00

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a follow up study of two sub-cohorts of Bedouin Arab children who were part of the infant feeding study of the NICHD and the Epidemiology Unit of the Ben Gurion University of the Negev study conducted between 1981 and 1983. Children from this original cohort will be identified through the school system and their height, weight and head circumference ascertained. In addition, information will be obtained through interview, both in the school and in the home to ascertain current socioeconomic status of the family, changes over time, as well as significant morbidity. The total sample will include 713 children and their mothers.

Funding for this study was obtained from the Israel Binational Science Foundation with Dr. Hundt listed as the Israeli principal investigator and Drs. Berendes and Forman as the U.S. principal investigators.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD00035-21 ERRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Structure and Function of Biologically Active Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Chen	Head, UMSPC ERRB, NICHD	
Others:	K.J.Catt	Chief	ERRB, NICHD
	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD
	L. Sun	IRTA Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

NCI-Navy Medical Oncology Br., NCI (B.E. Johnson)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Unit of Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the analysis, synthesis, and structure-function relationships of biologically active peptides. During this project year, (1) the design and synthesis of peptide antigens for immunological studies of the structure-function relationships of Angiotensin II type 1 (AT1) and GnRH receptors molecules, (2) antitumor activity of potent magainin analogues are the focus of studies. AII and GnRH receptors have the seven putative domains characteristic of G protein-coupled receptors. Based on amino acid sequences deduced from cDNA encoded for AT1a or AT1b receptor, four segments from all putative extracellular domains and three segments from three different putative cytoplasmic domains were selected, synthesized and conjugated to thyroglobulin. Different strategies were employed in order to achieve homogeneous linkage specifically at one of the termini, and to maximize exposure of epitopes in an attempt to obtain highly specific antibodies. Generation and characterization of antisera against these peptides are in progress. Using ligand affinity purified antibodies against Res. 1-26 of AT1b and Western blot analysis, two species with molecular sizes Mr=67 kDa and 44 kDa were found in a bovine adrenal membrane preparation, AT1a or AT1b transfected mouse adrenal tumor cell (Y1), and rat tissue homogenates. Similarly, four segments with length of 12-42 residues representing various putative domains of mouse GnRH receptor cDNA deduced amino acid sequence were selected, synthesized and conjugated to thyroglobulin for the purpose of raising antibodies against them. A peptide designated as GnRH4-42 was synthesized also for the purpose of the investigation of its ability to compete with the receptor for GnRH mediated G protein coupling and PKC activation. The antisera when available will have great utility for the study of AT1 and GnRH receptor tissue distributions, expression and inhibition of glycosylation and hormone associated signal transduction. Synthetic magainins A and G are highly potent in causing osmotic lysis of bacteria and protozoa. In addition to previously tested hematopoietic and small cell lung cancer cells (SCLC), these two magainin analogues were also tested for the growth inhibition of forty tumor cell lines of various types. They were most potent against melanoma, followed by hematopoietic, SCLC non-SCLC, renal, and least active against ovarian, colon and CNS cancer cells. These findings suggest that potent magainin analogues alone or in combination with chemotherapeutic agents may have potential for the development of anticancer drugs. In particular, a therapeutic agent based on a potent magainin analogue is worth exploring for the treatment for melanoma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD00146-18ERRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Studies of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Chen	Head, UMSPC ERRB, NICHD
Others:	J.L. Morell	Research Chemist ERRB, NICHD
	J.H. Brown	Research Chemist ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Biochemistry, New York University School of Medicine (S. Lee-Huang)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Unit of Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the structure-function relationship, determination of amino acid sequence as well as the identification and characterization of post-translational modifications of proteins. By an improved method for the purification of MAP 30 from the extract of *Momordica charantia* seeds, an additional component designated as MAP 30II was isolated and characterized. MAP 30II displays ribosome-inactivating activity, topoisomerase-like activity and inhibits HIV-1 infection as well as replication, as measured by syncytium formation and viral core protein p24 expression and HIV-associated reverse transcriptase in a manner similar to previously reported anti-HIV plant proteins MAP 30, TAP 29, GAP 31, DAP 32 and 30. Neither cytotoxicity to the host cells nor toxicity in mice can be detected. The complete amino acid sequence of MAP 30II has been elucidated for the first time. To elucidate the complete sequence, the reverse HPLC or SDS-PAGE purified fragments after trypsin, endoproteinase-Glu, BrCN or BNPS-skatole and endoproteinase-Lys C cleavages were analyzed by an automatic Edman degradation procedure. The sequence consists of 252 or 253 amino acid residues in a single chain without any intra-chain disulfide linkage, and is short from both ends as compared with the cDNA deduced 286 residue sequence. It has Asp as N-terminus and Gly or Asp as C-terminus. MAP 30II is homologous in sequence with α-trichosanthin which is an abortifacient. The primary amino acid sequence and the phosphorylation site of a heat- and acid-labile protein kinase substrate from rat brain were also determined. Since the N-terminus of the protein is blocked, the sequence was assembled from tryptic and BrCN cleaved fragments of ^{32}P-labelled, S-pyridinylethylated protein. The amino acid sequence aligns completely identical to the 78-residue sequence deduced from rat brain RC3 cDNA of a cortex-minus-cerebellum cDNA probe and is homologous to bovine neurogranin. From analyses of ^{32}P-labelled tryptic and BrCN cleaved peptides, only one phosphorylation site was identified as Ser^{36}. The sequence can be divided into three segments. Each has a distinctive feature: (1) Highly acidic in Res. 1-25; (2) The PKC phosphorylation site and calmodulin-binding domain in the middle; (3) A collagen chain-like structure in the last 25 residues. The understanding of these features permit us to design a specific PKC substrate AAAKIQASFRGHMARKKIK-amide for monitoring of PKC level in tissues and cells. The amino acid sequence analysis and C-terminal analysis of two metabolites derived from ACTH$^{1-39}$ led to the finding and characterization of mono- and di-peptidyl carboxypeptidases in bovine pituitary homogenate and secretory vesicles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00147-18-ERRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action of Peptide Hormones in Steroidogenic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.L. Dufau	Chief, SME	ERRB, NICHD
Others:	Y.-C. Koh	Visiting Associate	ERRB, NICHD
	J.C. Tinajero	Visiting Associate	ERRB, NICHD
	E. Buckzo	Adjunct Scientist	ERRB, NICHD
	A. Fabbri	Adjunct Scientist	ERRB, NICHD
	T. Ciampani	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contact for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome P450-17 alpha (CYP17), the key enzyme for the synthesis of androgen and glucocorticoid, catalyses two consecutive oxidation reactions in steroid metabolism at C-17 within either delta 5 or 4-pathway to produced the 17 alpha-hydroxylated intermediate (17 Beta-hydroxylase) and androgens products (17-20 lyase). Characterization of the rat CYP17 has revealed that the conserved domain between positions 343 and 363 carry charge aminoacids that contribute to lyase and hydroxylase activities. Arg 346 is essential for both delta 4 and 5-lyase activity and Arg363 is of importance to the hydroxylase reaction. Site directed mutagenesis studies have also determined species specific differences between rat and human/bovine/pig sequences for delta 4 versus delta 5-lyase activities. The substitution of Ile for Phe343 abolishes the enhancement of delta 4- over delta 5-lyase activity catalyzed by rat CYP17, and this substitution is the contributing factor to the absence of 4 delta lyse activity catalyze by human/bovine CYP17. The specific activation of the delta 4-lyase activity in the rat results from a stabilization involving stacking energies between the phenyl ring of Phe and the planar C-3 A ring of the delta 4 substrates. These findings emphasizing the physical separation of hydroxylase from lyase activity as well as the importance of domain 343-348 to lyase activity in CYP17 are of much relevance for the development of inhibitors of androgen formation for treatment of patients with prostate cancer, and for elucidation of the basis of certain forms of male pseudohermaphroditism. CRF, is an important antireproductive hormone of the testis and inhibits LH signal generation and androgen production. CRF exerts autocrine receptor mediated inhibitory actions in the Leydig cell and its secretion is stimulated by LH acting via a serotonin 5HT2 receptor. The secretion of CRF by the Leydig cell is stimulated by LH, acting via release of serotonin and autocrine activation of high affinity 5HT2 receptors. A low affinity 5HT2 receptor has also been demonstrated. This site has dual functions including:(a) stimulation of CRF secretion by 5HT or the 5HT2 agonist DOI [at pharmacological (μM) doses] and (b) autoreceptor properties that exert negative feedback regulation of 5HT release. Propranolol, the non-selective beta-adrenergic blocker, frequently used in the control of blood pressure in patients with mild to moderate hypertension and often associated with impotence, simulates CRF secretion via a serotonergic mechanism and inhibits gonadotropin-induced steroidogenesis in the Leydig cell. In contrast, selective beta-adrenergic blockers (atenelol, acebutolol or metoprolol) have no effect. Propranolol's effects occur through the antagonist action of the drug at the low affinity 5HT2 site. The blockade of this site by propranolol promotes the extracellular accumulation of 5HT, which binds to the high affinity 5HT2 sites and causes stimulation of CRF release and consequent inhibition of cAMP generation and steroidogenesis. Disruption of the serotonergic homeostatic mechanism by propranolol at the testicular level may be a contributing factor to the impairment of sexual function that commonly occurs during propranolol therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-18-ERRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Gonadal Receptors and Gonadotropin Biological Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, Title, laboratory, and institute affiliation)*

PI:	Maria L. Dufau	Chief, SME	ERRB, NICHD
Others:	C.-H. Tsai-Morris	Senior Staff Fellow	ERRB, NICHD
	E. Buckzo	Adjunct Scientist	ERRB, NICHD
	Z.Z. Hu	Adjunct Scientist	ERRB, NICHD
	X. Xie	NRSA Fellow	ERRB, NICHD
	Y. Geng	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904
University of Virginia, Department of Medicine, Charlottesville, VA (J. Veldhuis)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LHR gene consists of 11 exons separated by 10 introns that are all located within the coding region of the extracellular domain of the receptor. Exons 1-10 code for the extracellular region and exon 11 encodes the transmembrane and cytoplasmic domains and the 3' non-coding region. The several alternate truncated forms of the LHR conform to deletions of complete (exon 9) or partial exons (exon 11). It is likely that the hormone binding domain encompasses most of the region spanning exons 1-10, and that significant tertiary structured is involved. A TATAless promoter region was localized within the 173 bp of the 5'flanking region of the gene. Analysis of the 173-bp region revealed a minimal promoter domain between -1 and 137 bp with three SP1 sites and three consensus Initiator elements located at transcriptional start sites (TSS) -14, -19 and -33. Gel retardation studies showed a protein-binding DNA domain about 30 bp upstream of the TSS and an adjacent protein binding SP1 element that play a role in basal transcription. A second promoter domain (-120 to -173 bp) with a protein binding SP1 element was more prominent in non-expressing cells. Tissue-specific DNA binding proteins, observed only in expressing cells (M1, promoter I and M2, promoter II), are present in each of the promoter domains and may be important in tissue-specific regulatory events. A protein binding C-box element may contribute to differential mechanisms of derepression and repression in expressing and non-expressing cells. This domain may perform basal as well as inducible functions. Further studies have identified domains located upstream of the promoter region that repress transcription in expressing and non-expressing cells and an additional upstream inhibitory region (-1237 to -2056) in transfected non-expressing cells that may account for the normal lack of receptor expression in these cells. These studies indicate that a mechanism of derepression seems to accompany transcriptional initiation in the LH receptor gene and that gene expression may result from mechanisms that alter specific trans/trans interactions between upstream elements and the two promoter domains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00184-15 ERRB

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Regulation of Pituitary Hormone Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.J. Catt	Head, SHR	ERRB, NICHD
Other:	S. Stojilkovic	Visiting Scientist	ERRB, NICHD
	M. Tomic	Visiting Fellow	ERRB, NICHD
	L. Zheng	Visiting Fellow	ERRB, NICHD
	A. Sakai	Visiting Fellow	ERRB, NICHD
	J. Reinhart	IRTA Fellow	ERRB, NICHD
	L. Krsmanovic	Adjunct Scientist	ERRB, NICHD
	M. Cesnjai	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Lab of Cell Biology and Genetics, NIDDK (E. Rojas, M. Kukuljan)
Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH: Endocrinology and Reproduction Research Branch

SECTION: Section on Hormonal Regulation

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.5	7.5 0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cDNA encoding the gonadotropin-releasing hormone (GnRH) receptor was isolated by expression cloning from the αT3 gonadotroph cell line, using light emission in aequorin-injected *Xenopus* oocytes as a functional assay of expressed GnRH receptors. The receptor cDNA encoded a 327-amino acid protein with seven transmembrane domains that is unique among G protein-coupled receptors in lacking a C-terminal cytoplasmic domain. The GnRH receptor was found to be expressed not only in pituitary gonadotroph cells but also in the hypothalamic neurones from which GnRH is secreted. This finding suggested that the known feedback effects of GnRH on the hypothalamus can be exerted directly on the GnRH neurons. In accord with this, GnRH agonists were found to exert complex effects upon calcium signaling and secretory responses of immortalized GnRH neurons. These include an initial elevation and subsequent transient suppression of basal pulsatile secretion, followed by dose-dependent induction of less frequent but larger LH pulses and ultimately by single massive episodes of GnRH release. Thus, GnRH exerts autocrine actions on its own secretion, and promotes the synchronized discharge of GnRH in a manner that reflects the genesis of the ovulatory GnRH surge *in vivo*. Comparative studies on the actions of endothelin and GnRH in the pituitary gland revealed that these peptides operate through distinct receptors to activate the same signaling mechanisms for calcium release and influx. The transient stimulatory actions of endothelin may indicate its role as an accessory stimulus of LH release during the ovulatory surge of gonadotropin secretion. The signaling actions of GnRH were also analyzed in immortalized pituitary gonadotrophs, in which GnRH receptors activated the calcium signaling pathway by promoting both release from internal stores and influx through L-type calcium channels. In normal pituitary gonadotrophs, the mechanism of agonist-induced calcium oscillations was shown to include an essential action of Ins(1,4,5)P3 on the initiation of the oscillatory calcium response, as well on the control of its frequency. However, although the initial Ins(1,4,5)P3 concentration determines the frequency pattern of calcium signaling, maintenance of the signal does not appear to require continued elevation of Ins(1,4,5)P3. These and related studies have led to the proposal that the Ins(1,4,5)P3-sensitive channels in the endoplasmic reticulum are tonically inhibited by high intraluminal calcium levels, and that Ins(1,4,5)P3 surmounts such inhibition by promoting calcium discharge. At a critical level of calcium discharge, repetive calcium transients are generated by an autocatalytic mechanism that does not require a sustained increase in Ins(1,4,5)P3 production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HD00187-14-ERRB

PERIOD COVERED

October 1, 1992 to September 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Regulation of Cellular Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.-P. Huang	Head, SMR	ERRB, NICHD
Others:	F. Huang	Research Chemist	ERRB, NICHD
	C.W. Mahoney	Senior Staff Fellow	ERRB, NICHD
	K. Seki	Visiting Fellow	ERRB, NICHD
	L. Sheu	Visiting Fellow	ERRB, NICHD
	L.Shen	Visiting Fellow	ERRB, NICHD
	T. Sato	Guest Researcher	ERRB, NICHD
	M. Vieytes	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Biophysics, Kanazama Univ. School of Medicine, Japan (H. Higashida); Lab of Biochemistry, NCI, NIH (S.H. Wilson); Carnegie Institute of Washington, Baltimore, MD (S.L. McKnight)

LAB/BRANCH

Endocrinology and Reproductive Research Branch

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
7	7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C (PKC) has emerged as a pivotal regulatory element for the regulation of many cellular functions. Multiple PKC subspecies have been identified by molecular cloning, however, the functional role of each of these enzymes is largely unknown. These enzymes were found to have distinct tissue, cellular, and subcellular distributions and were differentially expressed during development. These kinases are stimulated by Ca2+/phospholipid/diacylglycerol (DG). DG derived from phosphoinositides are more potent activators than those derived from phosphatidylcholine. Since activation of PKC results in the phosphorylation of target proteins, we have isolated several PKC substrates from rat brain for analysis of their physiological functions. Among them, neurogranin (Ng), neuromodulin (Nm), and MARCKS are the most prominent PKC substrates in the brain. Ng and Nm bind calmodulin (CaM) with high affinity in the absence of Ca2+ whereas MARCKS does so in the presence of Ca2+. Phosphorylation of these proteins by PKC reduces their affinities toward CaM and thus release CaM for the CaM-dependent enzymes. In addition to the phosphorylation-induced effects, oxidation of Ng by peroxide or nitric oxide also reduces the affinity of this protein for CaM. Dephosphorylation of Ng and Nm are catalyzed by protein phosphatase 1, 2A, and calcineurin; however, MARCKS is dephosphorylated more efficiently by protein phosphatase 1 compared to phosphatase 2A and calcineurin. These latter two phosphatases selectively recognize a set of sites in the PKC-phosphorylated MARCKS, whereas phosphatase 1 dephosphorylates all the PKC phosphorylation sites. In addition to the aforementioned PKC substrates, four novel proteins have been isolated as potential substrates of PKC. Complete amino acid sequence of one of them, named PKCS-38, has been determined; this protein has a structural feature resemble the helix-turn-helix family of DNA-binding proteins and contains two PKC phosphorylation sites and one potential cell cycle-regulated cdc2 kinase site. Molecular cloning of the PKCS-38 cDNA is in progress. The genomic structures of CNS-specific PKC gamma and Ng were analyzed for the purpose of defining tissue-specific and development-regulated expression of these two proteins. Multiple nuclear protein binding elements in PKC gamma gene have been identified by footprint and gel mobility shift assays. A potential negative regulatory element-binding protein, its expression is inversely related to the expression of PKC gamma, was identified in fetal rat brain nuclear extract. A similar binding element has also been identified at the same position in the 5'-flanking region of the Ng gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00193-08 ERRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Angiotensin II Receptors and Activation Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.J. Catt	Head, SHR	ERRB, NICHD
Others:	T. Balla	Visiting Scientist	ERRB, NICHD
	A.J. Baukal	Chemist GS-12	ERRB, NICHD
	K. Sandberg	Sr. Staff Fellow	ERRB, NICHD
	H. Ji	Visiting Fellow	ERRB, NICHD
	L. Hunyady	Adjunct Scientist	ERRB, NICHD
	Y. Tian	Adjunct Scientist	ERRB, NICHD
	S. Nakanishi	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Physiology, Semmelweis University Medical School, Budapest
Laboratory of Biochemistry, NHLI, NIH (S.G. Rhee)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER
7.5	7.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the receptors and signal transduction mechanisms of angiotensin II (AII) and ACTH were performed in adrenal glomerulosa cells and *Xenopus laevis* oocytes. In the adrenal gland, angiotensin II (AII) was found to act through AT1 receptors to induce the expression of several early growth response genes, including c-fos, c-jun, JunB, and Krox 24, in glomerulosa cells. These actions of AII on growth-related cellular oncogenes were found to be mediated by elevation of cytoplasmic calcium and activation of protein kinase C, both of which occur during stimulation of the adrenal glomerulosa cell by AII. These studies also revealed a major difference between the signaling requirements of the secretory and "nuclear" responses in glomerulosa cells in that unlike the former, the latter did not require the presence of extracellular Ca2+ and only a brief exposure to the hormone could elicit the full early gene response. It was also shown that the presence of extracellular Ca2+ has an inhibitory action on the early gene response that slows down the kinetics of the transient c-fos expression, and reveals a dual role of Ca^{2+} in the regulation of c-fos mRNA levels. The early gene responses evoked by AII were not associated with a mitogenic response in short-term cultures of adrenal glomerulosa cells. However, mitogenic responses to AII became evident when cells were maintained in culture for several days and were accompanied by increases in cell number. These findings, and related studies in stably transfected adrenal Y1 cells expressing AII receptor subtypes (AT1A and AT1B), are providing new information about the signaling mechanisms by which AII controls not only the steroidogenic and secretory activities but also the growth and proliferation of the adrenal glomerulosa cell. In further studies on the structure-function properties of the AT1 receptor, the cDNA encoding a calcium-mobilizing amphibian AII receptor was cloned from the *Xenopus laevis* myocardium. Previous work in the laboratory has shown that the amphibian AII receptor possesses unique binding and activation properties, and does not recognize the non-peptide antagonists that act on the mammalian AII receptor subtypes. In keeping with this, the cloned amphibian receptor has about 60% amino acid similarity with the known mammalian AT1 receptors, with many differences in regions likely to participate in the binding site for AII and its analogs. Knowledge of the structure of this unique AII receptor will be utilized in studies on mutant forms of the mammalian AT1 receptor, to identify the residues and sequences that are involved in the ligand binding domains of the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00194-05 ERRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Steroid Biosynthesis and Metabolism in the Mammalian Adrenal Cortex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	C.A. Strott	Head, SACB	ERRB, NICHD
	Y.C. Lee	Chemist	ERRB, NICHD
	W.J. Driscoll	Senior Staff Fellow	ERRB, NICHD
	K. Komatsu	Visiting Fellow	ERRB, NICHD
	C. Park	Visiting Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Armed Forces Radiological Research Institute (Dr. Mark Whitnall)
Section on Molecular Structure and Protein Chemistry, NICHD (Dr. Hao-Chia Chen)
Clinical Neuroscience Branch, NIMH (Dr. Brain Martin)
Laboratory of Biophysical Chemistry, NHLBI (Dr. Henry Fales)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20893

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The sulfoconjugation of steroids is an important metabolic route for these compounds. Steroid sulfonation appears to have multiple biochemical and functional implications: it increases steroid polarity and water solubility influencing transportability, it can function as an activating or inactivating mechanism and it can function as a trapping or storage mechanism. One of the most active tissues to sulfonate steroids is the adrenal cortex where its biological significance is not well understood. Therefore, to shed light on steroid sulfonation in the mammalian adrenal cortex, current research effort in the laboratory is focused on the family of sulfoconjugating enzymes known as sulfotransferases.

Estrogen sulfotransferase (EST) cDNA and the 5'-flanking region of the EST gene have been cloned. The EST promoter contains motifs related to steroid response elements (SRE) and sequences analogous to binding sites for the SF 1 transcription factor (coordinately regulates expression of steroidogenic enzymes) and the AP-1 family of transcription factors (jun/fos). Estradiol stimulated EST promoter activity in a dose-dependent fashion; tamoxifen inhibited the estradiol effect, suggesting involvement of the estrogen receptor. Additionally, gel mobility shift assays detected nuclear proteins that specifically bind to SRE constructs.

Two physically distinct hydroxysteroid sulfotransferases (HST) demonstrating substrate specificity with respect to orientation of the A ring 3-hydroxyl group were purified to homogeneity and peptides sequenced. The 3beta-HST has an apparent Mr of 33 kDa, while the 3alpha-HST is slightly smaller at 32 kDa. This is the first definitive study demonstrating the existence of discrete HSTs that exhibit substrate specificity based on the configuration of the 3-hydroxyl group. An unexpected observation was that 3alpha-HST constitutes a remarkable 13% of the total adrenal soluble protein. The predominance of 3alpha-HST in reticularis suggests a role of some significance for this enzyme in this cell type. The 3alpha-HST cDNA has been cloned and expressed.

Immunocytochemical localization demonstrated EST in fasciculata and reticularis cells, while HSTs were present only in reticularis cells. Electron microscopic analysis revealed that EST was present in nuclei with sparse labeling throughout the cytoplasm; HST labeling was intense in the smooth endoplasmic reticulum but absent from nuclei. Peculiar inclusion bodies observed only in reticularis cells were strongly labeled for both EST and HST. The high concentration of EST in nuclei suggests that EST might play a role in modulating estrogenic regulation of gene expression in ACTH-responsive cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00323-13 EB

PERIOD COVERED
October 1, 1992 through November 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
District of Columbia Perinatal Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.W. Berendes	Director	OD, DESPR, NICHD
Other:	L.C. Cooper	Nurse Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The District of Columbia (D.C.) Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to black mothers residing in D.C. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race-matched normal weight infant (≥2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. If the hospital medical record, however, did not contain adequate prenatal information, arrangements were made to abstract this information from private and public physicians' offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). It was necessary to re-key all of the data originally submitted by SRA Technologies. One hundred percent of the data have now been keyed. Manuscripts are now in the process of being prepared for submission to peer reviewed journals.

This project was terminated.

001

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00325-12 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION
Pediatric Epidemiology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Epidemiology Branch (DESPR) has conducted a case-control study in Illinois and California to determine whether the use of periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect have been ascertained through perinatal networks, vital records, and other sources and were matched to two controls on maternal race and geographic locale. One control is a mother with a normal pregnancy, and the other the mother of an infant with a fetus with a major health problem other than a neural tube defect. Cases and controls were interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. A second major objective was to determine whether or not fertility drug use is associated with neural tube defects. Data on infertility and the use of ovulation-inducing drugs were collected during the same interview. Additional analysis has examined the effects of extreme obesity.

002

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00331-10 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes In Early Pregnancy (DIEP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Cornell Univ.Med.Center, NY (L.Jovanovic); Brigham and Womens Hosp., Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ.of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ.of Washington, Seattle, WA (R.Knopp)

LAB/BRANCH
Epidemiology Branch

SECTION
Pediatric Epidemiology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Diabetes in Early Pregnancy Project was designed 1) to examine the relationship between maternal diabetic control during organogenesis and malformations in the offspring, and to identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) to compare early fetal loss rates in women with diabetes and in non-diabetic control subjects. We found that diabetic women who came into care before the period of organogenesis achieved better results than those who came in later; but their results were still poorer than for non-diabetic control subjects. Differences in maternal glucose levels during organogenesis did not explain the malformations in the offspring of the women who were followed throughout pregnancy. These results suggest that women who enter late (and were not under medical supervision during organogenesis) probably had poor control. This resulted in malformations due to hyperglycemia or related factors. The diabetic group entering early strongly suggests that other teratogenic mechanisms were present. Regarding early fetal losses, we found that diabetic women in good metabolic control were at no higher risk for spontaneous abortion than control women; the risk of loss increased dramatically as diabetic control worsened; and the overall risk of losing a pregnancy was lower than expected, only 16%. Since these primary analyses were completed, a number of related studies have been completed (see previous reports). At present, issues relating to minor malformations, metabolic factors, and retinopathy are being addressed. Blood specimens at the Sansum Foundation have been assayed for glycosylated protein to examine the relationship between intermediate term control and malformations although funding problems have slowed progress. The National Eye Institute has completed analysis of retinal photographs taken at the beginning and end of pregnancy to assess the effect of pregnancy on retinopathy. The University of Tennessee has measured blood levels of anti-sperm antibodies and anti-phospholipid antibodies in relation to spontaneous abortion.

005

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 0334-10 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS (if any)
Office of the Director, DESPR, NICHD (H.W.Berendes); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The original description of the correlation between birthweights of mothers and their infants was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In ensuing studies, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small-for-gestational age infant as were women who weighed 4000-4499 grams at birth, but low birth weight women were less than twice as likely to have a preterm infant. A group of Swedish women, born from 1955 to 1965, was next studied. Women who themselves were small-for gestational age at birth were at increased risk of giving birth to both small-for-gestational age and preterm infants. Women who were preterm at birth were not at increased risk of either outcome.

Analysis of data from girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently underway in order to examine their reproductive histories. Small-for-gestational age, preterm and control girls have been located and interviewed. Hospital records of their confinements have also been retrieved.

003

PHS 6040 (Rev. 5/82)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00340-10 EB

PERIOD COVERED
October 1, 1992 through November 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Women's Life Styles during Pregnancy Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: L.C. Cooper Nurse Epidemiologist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Office of the Director, DESPR, NICHD (H.W. Berendes)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
A contract to characterize behavior and lifestyle differences among pregnant women of different ethnic groups which are known to differ in their rates of low birth weight was awarded to Columbia University and to Northwestern University. The overall goal of this project is to define risk factors affecting birth outcomes in pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, Dominicans, and Whites. The work scope of the contract included the development of an extensive questionnaire by a multi-disciplinary team of experts, pretesting of the interview instruments, interviewing pregnant women from the six groups noted above, ascertaining birth outcomes, and preparing an edited data tape of all responses.

This project was terminated.

01?

PHS 6040 (Rev. 5/82)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00361-07 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Child Health Supplement to the 1988 National Health Interview Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
Other:	A. Trumble	Survey Statistician	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*
HLB, CRMC, NICHD (P.C.Scheidt); National Center for Health Statistics (A.Moss); Bar Ilan University (Y.Harel); Centers for Disease Control (D.Jones); Albert Einstein College of Medicine (P.Bijur); FDA (R.Bright)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This survey provides population-based data on a nationwide representative sample of 17,200 children. Subjects include child care, family relationships, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It provides data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey was conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year with support and coordination by the NICHD. Data analysis is being conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00363-06 EB

PERIOD COVERED
October 1, 1992 through June 1, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NICHD Smoking Trial of Pregnant Women (STOP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: L.C. Cooper Nurse Epidemiologist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Office of the Director, DESPR (H.W. Berendes)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The STOP Project is a randomized clinical trial designed to evaluate the effectiveness of a behavioral intervention program to encourage cessation of smoking by pregnant women attending routine prenatal care in private physician settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who report smoking at their first prenatal care visit. Physicians will be recruited to volunteer to take part in this randomized study.

There were to be two major phases to the STOP Project - a pretest phase and a formal trial with a built-in pilot study. The objectives of the pretest phase were: 1) to develop the protocol for the STOP study; 2) to develop all study materials (e.g., pamphlets, study forms, manual of operations); 3) to recruit private physicians who will assist in finalizing the study protocol and educational materials; 4) to assist in the development of all quality control procedures; and 5) to train the contractor selected to implement the formal trial in all aspects of the study. At this time, the pretest has been completed and all educational materials for the study have been completed and approved by the physicians and staff participating in the pretest phase. Two contractors, the University of Maryland and Westat, Inc., were selected to execute the pilot and formal trial for the STOP Project. The contract was signed and went into effect on May 15, 1991. The project is presently being piloted in Baltimore, Maryland.

This project was terminated due to findings in the pretest indicating that cooperation by private physicians' offices would pose a major obstacle to successful conduct of the main trial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00369-05 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adverse Perinatal Events and Subsequent Injury-related Death

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIMH (M. Farmer)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.60	0.10	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several published studies have indicated that children experiencing asphyxia during the perinatal period are at increased risk of subsequent adolescent suicide. However, these studies used retrospectively ascertained data and were unable to control for the adverse social conditions that often accompany perinatal difficulties.

In the first phase of this project, the names of the approximately 55,000 children who were born during 1959-66 as subjects in the Collaborative Perinatal Project were computerized. During the second phase, the computerized names and other appropriate identifying information will be submitted to the National Death Index, and all subjects who have died will be identified. In the third phase, the death certificates of these subjects will be obtained and cause of death recorded. Since the Collaborative Project collected extensive data about the subjects' prenatal, perinatal, and childhood histories, it will be possible to study prospectively the relatic :hip between adverse perinatal events and subsequent risk of ;eath, as well as cause of death.

01?

NOTICE OF INTRAMURAL RESEARCH PROJECT | Z01 HD 00373-05

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Calcium Supplementation in Pregnancy for the Prevention of Preeclampsia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.J. Levine	Expert (Epidemiology)	EB, DESPR, NICHD
Others:	R. DerSimonian	Mathematical Statistician	BMSB, DESPR, NICHD
	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
	E. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

University of Alabama (J.C.Hauth); Case Western Reserve U. (P.M.Catalano); Univ. of New Mexico (L.B.Curet); Oregon Health Sci.Univ. (C.Morris); Univ. of Tennessee (B.M.Sibai); The Emmes Corp. (C.L.Carter); Biomedical Research Inc. (J.Leaf)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The results of six clinical trials suggest that supplemental calcium may prevent preeclampsia. Methodologic problems, however, limit the credibility of the results and their generalizability to other patient populations. Moreover, none of the trials has reported the outcome of systematic surveillance for urolithiasis, an important possible complication of treatment. In response to the need for a definitive evaluation of the effects of calcium supplementation, NICHD is conducting a trial at five U.S. university medical centers. Healthy nulliparous patients are randomly assigned to receive either 2 g ingested supplemental calcium daily (n=2250) or placebo (n=2250) in a double-blind study. Medication is administered beginning between 13 and 21 completed weeks of gestation and continued until the termination of pregnancy. Monitoring for the major study endpoints - pregnancy-associated hypertension and proteinuria, preeclampsia, eclampsia, and HELLP syndrome - and for urolithiasis will be systematic, standardized, and thorough. It will include measurement of blood pressure, proteinuria, and hematuria at uniformly scheduled prenatal clinic visits and surveillance for hypertension and proteinuria during labor, delivery, and the first 24 hours postpartum. CPEP should have adequate statistical power to detect a reduction of 43 percent in preeclampsia risk in the calcium group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00378-04 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Mortality of Infants Weighing Less than 750 Grams

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.D. Overpeck Epidemiologist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NCHS (K.Prager); NICDD (H.J.Hoffman)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1		0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The 1983 birth cohort of the National Linked Birth/Infant Death Data Set was analyzed to document the contribution of live born infants weighing less than 750 grams to the U.S. infant mortality rate. Previously, linked birth and death certificates for the total U.S. were not available, preventing assessment of the mortality experience of these least viable infants. This information is necessary in order to target interventions to reduce the infant mortality rate. The data set represents an intensive effort by NCHS to obtain data from all states and reporting areas. The first analysis was completed and published by the American Journal of Public Health in 1992 with subsequent work published in 1993. Project extended to FY'93 for additional analysis to respond to Letters to the Editor.

022

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00379-04 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Analysis from the Vaginal Infections and Prematurity Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD
Other:	J.S. Read	Epidemiology Staff Fellow	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
PAMA, CRMC, NICHD (R.Nugent); NIAID (M.F.Cotch); Research Triangle Institute (A.V.Rao); Columbia University (J.Regan)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS.	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Vaginal Infections and Prematurity (VIP) Study, jointly sponsored by NICHD and NIAID, is a prospective study designed to investigate the relationship between genital tract colonization with various microorganisms and the subsequent development of preterm birth. It also incorporates a clinical trial of erythromycin to prevent preterm birth among women colonized with *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and group B streptococcus. In addition, the VIP collected a wealth of additional data on a variety of factors possibly linked to pregnancy outcome. This project will analyze these factors.

Analyses undertaken to date include the predictive value of vaginal Gram stain in the identification of group B streptococcus, the descriptive epidemiology of group B streptococcal carriage, and the relationship between reported physical activity and preterm birth. Questions under analysis include the effect of group B streptococcal colonization on pregnancy outcome, the effect of treatment with erythromycin on pregnancy outcome among women colonized with group B streptococci, the association between sexual intercourse during pregnancy and preterm birth among colonized with different genital microorganisms.

024

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00382-03 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cocaine and Marijuana Use During Pregnancy and Pregnancy Outcome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Office of the Director, DESPR (H.W.Berendes); PAMA, CRMC (R.Nugent); Research Triangle Institute (M.Cotch); Packard Foundation (P.Shiono); University of Utah (D.Rollins)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The relationship between illicit drug use and pregnancy outcome has been controversial. Self-reported drug use is notoriously inaccurate, and selective screening of blood and/or urine often results in biased ascertainment of drug use, as the reason for screening may be as important a risk factor as the drug use itself. There have been a few systematic large-scale studies of drug use at specified times during pregnancy in a defined cohort of women, and even fewer that have collected detailed data on other risk factors for adverse pregnancy outcome.

In this project, serum obtained from approximately 7,500 women on entry to the Vaginal Infections and Prematurity Study (23-26 weeks) were screened for marijuana and cocaine metabolites. In addition, serum obtained at delivery of low birth weight, preterm and a random sample of term infants was screened, as was serum obtained at 31-36 weeks from a random sample of uncomplicated pregnancies and serum obtained from women experiencing abruptio placenta and placenta previa. The Vaginal Infections and Pregnancy Study collected extensive data on a variety of confounding variables, and enabled the most thorough analysis yet of the effect of cocaine and marijuana use on pregnancy outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00383-03 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analyses of Data from the Collaborative Perinatal Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
University of California at San Francisco (T.Newman); NEI (E.Chew); University of Maryland (D.Nagey); Packard Foundation (P.Shiono)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.30	.30	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Collaborative Perinatal Project (CPP) represents a rich data set for the investigation of a variety of topics related to maternal and child health.

Currently active areas of investigation include the association of neonatal hyperbilirubinemia and neurodevelopmental outcome at age 7, the descriptive epidemiology of strabismus, the association between elevated maternal serum levels of alpha fetoprotein at 14-20 weeks and subsequent stillbirth, and the risk of childhood cancer following neonatal vitamin K exposure.

030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00384-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Field Trial of Oral Cholera Vaccines in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Johns Hopkins School of Public Health (D.Sack); International Center for Diarrheal Disease Research, Bangladesh

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project entails continuing analyses of data derived from a large-scale, placebo-controlled field trial of B subunit-killed whole cell (BS-WC) and killed whole cell-only (WC) oral vaccines against cholera, conducted between 1985-90 in the Matlab field studies area of the International Center for Diarrheal Disease Research, Bangladesh. The trial enrolled ca. 89,000 subjects for study. Analyses of the first three years of follow-up revealed that each vaccine conferred ca. 50% protection against cholera episodes detected among patients seeking care at medical facilities.

Additional work is ongoing to determine whether antecedent infection by Helicobacter pylori, a known cause of persistent hypochlorhydria, is a risk factor for cholera in Bangladesh. This study is evaluating serological evidence of H. pylori infection in cholera cases and community controls assembled during the field trial.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00385-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiology of Rotavirus Infections in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
James Gamble Institute for Medical Research, Cincinnati, OH (R.Ward)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project tests the hypothesis that humoral immunity to rotavirus infection, assessed in serum, is associated with the risk of rotavirus diarrhea in children aged under 2 years who reside in rural Bangladesh. Cases of rotavirus diarrhea were assembled from population-based, comprehensive surveillance of treated episodes of pediatric diarrhea in Matlab, Bangladesh during 1985-86. All rotavirus diarrhea episodes were serotyped with monoclonal antibodies after cultivating rotavirus isolates. Controls were similarly aged childred randomly selected during four surveys of the Matlab community undertaken during 1985-86. Serological correlates of natural immunity to rotavirus diarrhea were assessed by contrasting acute-phase sera collected from the cases and sera from controls. Case-control comparisons revealed that: 1) serum IgG antirotavirus antibodies were correlated inversely with the occurrence of rotavirus diarrhea, suggesting a protective relationship; but 2) protective associations were not serotype-specific.

A second goal is to evaluate whether breast feeding is associated with a reduced risk of rotavirus diarrhea in children under the age of 2 years. Using a case-control sampling strategy analagous to that described for the seroepidemiological assessment, comparisons of cases and controls for antecedent histories of breast feeding revealed that: 1) breast feeding was associated overall, with a reduced risk of rotavirus diarrhea, due to the protection conferred by exclusive but not partial breast feeding; but 2) after infancy, the direction of the relationship became reversed, with a higher risk of rotavirus diarrhea among breast-fed than non-breast-fed children. Future analyses will assess whether the protection observed during infancy is correlated with antirotavirus and neutralizing antibodies in breastmilk.

037

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00386-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of a Water-Sanitation Intervention in Egypt

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
UNICEF, Egypt

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this field project is to evaluate the impact of a combined water-sanitation intervention on the occurrence of pediatric diarrhea and other targeted outcomes in a study population residing in the Assiut governorate of Upper Egypt.

20 villages, which together included a population of approximately 10,000 persons, were randomly assigned to receive the intervention (N=10) or no intervention (N=10). The intervention consisted of provision of India Mark II tubewells and household latrines, together with an educational package stressing personal hygiene and water behaviors. To assess the impact of the intervention, all families in these villages were followed with logitudinal surveillance during a one-year follow-up period.

Although the baseline incidence of diarrheal episodes (per 1000 person-days of follow-up) in children under three years of age was similar in intervention (29) and control (29) communities, after zero-time rates of diarrhea decreased 17% ($p<.0001$) in the intervention relative to the control communities. Protective associations were slightly higher for blood diarrhea (25%, $p<.05$) and persistent diarrhea (26%, $p<.0001$), suggesting that the intervention might have reduced not only the risk of diarrhea but also the severity of diarrhea. In contrast, no impact on the occurrence of respiratory infections was evident, indicating that the decline of diarrhea in the intervention communities was not likely to be due to ascertainment bias. It was of particular interest that intervention communities exhibited a marked increase of per capita water consumption, but that the new pumps continued to produce water that was contaminated by fecal coliforms. There data suggest that the salutory effect of the intervention may have been mediated, in part, by increased water consumption, though the water itself was fecally contaminated. Analyses will continue in FY94.

040

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00387-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sexual Intercourse during Pregnancy and Preterm Delivery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Staff Fellow	EB, DESPR, NICHD
Other:	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.10	.10	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from the Vaginal Infections and Prematurity (VIP) Study were analyzed to determine the relationship between the frequency of sexual intercourse during pregnancy and preterm delivery and how this relationship is affected by vaginal microbiology. The population consisted of women seeking prenatal care from 23 to 26 completed weeks of pregnancy at one of seven clinical sites (Columbia University and Harlem Hospital, New York; University of Washington, Seattle; University of Oklahoma, Oklahoma City; University of Texas, San Antonio; Tulane and Louisiana State Universities, New Orleans) from 1984 to 1989. The women were enrolled in a standardized protocol and upon entry (23-26 weeks' gestation) were interviewed to obtain demographic, social, and sexual histories and underwent physical examinations, at which time a standardized set of genital culture specimens were obtained.

The association between frequency of sexual intercourse and preterm birth was studied among women with and without various genital microorganisms.

043

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00388-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Birth Weight and Infectious Disease Mortality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from the Collaborative Perinatal Project were analyzed to determine whether moderately low birth weight, singleton babies without congenital anomalies were at increased risk of infectious disease mortality. In this study, children with birth weight of 1500 to 2499 grams were compared to children with birth weight ≥2500 grams with regard to infectious disease mortality in the late neonatal and postneonatal periods of infancy as well as in childhood. We hypothesized that birth weight was inversely related to the risk of infectious disease mortality, with the strength of the relationship decreasing with increasing age at death and varying according to whether low birth weight was a manifestation of preterm delivery or intrauterine growth retardation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00389-02 EB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Epidemiology of Pediatric Shigellosis in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

International Center for Diarrheal Disease Research, Bangladesh; Johns Hopkins University (F.Ahmed)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0

CHECK APPROPRIATE BOXIES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this field project is to evaluate the epidemiological patterns and determinants of pediatric shigellosis occurring among residents in a research field site in rural Bangladesh who have had a documented recent exposure to a neighborhood resident having Shigella dysentery.

The project entailed assembly of 1934 children under 5 years of age, from 1335 families residing in 240 neighborhoods having a resident who presented for treatment of documented Shigella dysentery within 1 week of the initiation of active neighborhood surveillance. Following assembly of these children, each was followed with intensive active surveillance for one month. This surveillance included, at baseline, sociodemographic characterization, ascertainment of dietary and anthropometric status, and measurement of serum vitamin A. Subjects were visited according to a systematic schedule to ascertain symptomatic histories related to diarrhea, and to collect fecal specimens for microbiological characterization.

Analyses of the data thus far have demonstrated that the occurrence of Shigella diarrhea was related to age (being highest in the third year of life), nutritional status (being higher in stunted children), season (being lowest during the monsoon season in the summer months), and breast-feeding status (being lower among breast-fed children, even up to 36 months of age). The presence of a latrine was not related to the risk, due to the fact that the presence of an unsanitary hanging latrine elevated the risk, while the presence of a sanitary slightly depressed the risk. Interestingly, the protective effect of breast feeding was almost entirely attributable to the increase in risk of shigellosis which occurred during the initial 3 months following cessation of breast feeding.

Analyses are now in progress to determine the frequency and determinants of the progression of Shigella diarrhea from acute to chronic form and to assess the role of vitamin A deficiency as a risk factor for acute and persistent shigellosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00390-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Risk Factors for Stillbirth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	S. Cnattingius	Visiting Scientist	DESPR, NICHD

COOPERATING UNITS *(if any)*
NCHS (J.Kiely)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Advanced maternal age, nulliparity, and cigarette smoking are established risk factors for stillbirth. We used data from the Medical Birth Register of Sweden, 1983-1989, to examine the interactions between these risk factors and gestational age, and to explore possible clinical mediators of these relationships. All singleton pregnancies of at least 28 weeks gestation to nordic citizens at least 20 years old were included in the analysis. We found significant interactions of both maternal age and smoking with gestational age, and these interactions had different patterns: over the course of the third trimester the age-related risk of stillbirth increased, whereas the smoking related risk decreased. The higher risk in nulliparas remained constant. The elevated stillbirth risk in smokers appeared to be explained entirely by the higher incidence of intrauterine growth retardation and placental complications in smokers. However, the increased risk in older women and nulliparas was not explained by any of the pregnancy complications examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00391-02 EB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Risk Factors for Placental Abruption

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J.L. Mills	Chief, PES	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To study risk factors for placental abruption, we used data from the Kaiser Permanente Birth Defects Study, which was conducted by the National Institute of Child Health and Human Development between 1974 and 1978. This was a prospective cohort study which included 30,681 members of the Kaiser Permanente Health Plan who registered for prenatal care at one of 13 Kaiser facilities in Northern California. Demographic and social information was collected from these women at the first prenatal visit, and data about pregnancy complications and neonatal outcomes were obtained by review of the patient records. In our analysis, we found that important maternal risk factors for abruption included maternal age, less than 12 years of education, and cigarette smoking. Abruption was also significantly associated with intrauterine growth retardation and fetal malformations, and particularly with congenital heart defects.

This project was terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00392-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fatal Injuries to U.S. Infants, 1983-1986

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
	H.W. Berendes	Director	DESPR, NICHD
	A.C. Trumble	Computer Specialist	CSB, DESPR, NICHD
	E.R. Toretsky	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Center for Health Statistics linked birth and death records for U.S. infants provides the first opportunity to assess the demographic, socioeconomic and prenatal risk factors associated with deaths due to injuries in infants less than one year of age. U.S. studies of childhood deaths have been limited to the extremely sparse information available on standard death certificates. Both descriptive and risk factor analysis of infant deaths for 1983-1987 are being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00393-02 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Trends in Death Rates from Drowning among Children, 1971-1988

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS (if any)
Johns Hopkins University (G.Smith)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Age, race and sex specific trends in childhood drowning rates in the United States, from 1971 through 1988 were studied. This study is the first to compare age specific trends by race. Previous studies have shown two distinct populations to be at greatest risk of drowning, white toddlers and adolescent males (particularly black adolescent males). Results showed that, while there have been marked decreases in drowning rates in both white and black adolescent males, there have been only slight decreases in drowning rates among toddlers. In the white population toddlers have surpassed adolescents as the age group at highest risk of drowning. In the black population adolescent males remained the group at highest risk throughout the time period.

The interventions to prevent drowning vary with age. The results from this study not only help us to see the effectiveness of past interventions but also should aid us in targeting future interventions.

057

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00394-02 EB

PERIOD COVERED
October 1, 1992 through July 9, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gestational Age-Birth Weight Specific Mortality: Twins vs Singletons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: W.F. Powers Staff Fellow EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NCHS (J. Kiely); NIAID (M.G. Fowler)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The investigators have been asked to write a chapter for a textbook tentatively titled The Obstetric Management of Multifetal Pregnancy. Dr. Powers has recently written a thesis for his Masters Degree. The subject of the thesis was prophylactic interventions in twin pregnancy, and as part of the literature review, data from NCHS on the rate of increased survival of twins with increasing gestational age were reviewed. Dr. Kiely has reported on birth weight specific mortality of twins as ascertained from the New York City fetal death, live birth and infant death files. Dr. Fowler has compared survival rates of black and white twins and singletons from the 1960 and 1983 U.S. Linked Birth/Infant Death Data Sets from NCHS. The authors have consolidated these reports and expanded them into a chapter for the above mentioned text.

This project is completed.

050

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00395-02 EB

PERIOD COVERED
October 1, 1992 through July 9, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Relationship between Air Leak and Chronic Lung Disease in VLBW Infants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.F. Powers	Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The neonatal literature disagrees about the true relationship between air leak (pneumothorax or pulmonary interstitital emphysema) which occurs in mechanically ventilated very low birth weight infants (VLBW, 501-1500 grams) and chronic lung disease (CLD, need for supplemental oxygen at 28 days of age). A data set collected from a prospective cohort of VLBW infants born in three central Illinois hospitals was analyzed to define the strength of this relationship more precisely. Infants ventilated from the first day of life comprised the study group. Some develop air leak while being ventilated. The purpose of this study was to assess the incremental risk of adverse outcome (death or development of CLD) associated with development on an air leak in the first twenty-seven days of life.

This project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00396-02 EB

PERIOD COVERED
October 1, 1992 through July 9, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Risks Confronting Twins: A National Perspective

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: W.F. Powers Staff Fellow EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NCHS (J.Kiely)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from the Linked Birth/Infant Death files prepared by the National Center for Health Statistics are being used to calculate race-specific relative risks and population attributable risks for five adverse outcomes (low birth weight, very low birth weight, neonatal death, post-neonatal death, and infant death) in twin pregnancy. The role of the former two intermediate outcomes in explaining the later three outcomes will also be assessed. Because the data set used is population-based for the entire nation, the risk measures reported will be free from referral biases that frequently distort risk estimates that originate from teaching or referral hospitals.

This project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00801-18 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies Based on the Medical Birth Registries of Norway and Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
Other:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIDCD (H.Hoffman); Univ. of Bergen, Norway (P.Bergsjo, L. Irgens); Univ. of Trondheim and Natl.Inst.of Publ.Hlth., Oslo, Norway (L.Bakketeig, A.Arntzen); Uppsala Univ. (G.Lindmark, O.Meirik).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS.	PROFESSIONAL:	OTHER.
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
These studies have focused on: 1) the relation of the quality of medical care to the risk of perinatal death, 2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, 3) perinatal mortality in relation to order of birth and size of sibship, 4) epidemiologic risk factors for preterm birth, 5) epidemiologic risk factors for small-for-gestational age births, 6) contribution of multiple births to perinatal mortality rates, 7) size at birth as measured by head circumference, crown-heel length, and birth weight in relation to gestational age and perinatal mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00861-11 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: A.A. Herman Visiting Scientist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIDCD (H.Hoffman); U.Trondheim, Norway (G.Jacobsen, L.Bakketeig); U. Bergen, Norway (P.Bergsjo, T.Markestad); U.Uppsala, Sweden (G.Lindmark, G.Ahlstein); Vanderbilt U. (G.W.Reed); U. Alabama at Birmingham (R.Goldenberg, K.Nelson)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a prospective study of risk factors associated with intrauterine growth retardation. Recruited were pregnant women before 17 weeks gestation at the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala) for the period of January 1986 through June 1988. The children are followed for five years to monitor physical growth and behavioral development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00872-08 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Associated with Premature Births: Missouri Follow-Back Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
Other:	H.W. Berendes	Director	OD, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIDCD (H.J.Hoffman); State Center for Health Statistics, Missouri Dept. of Health (G.Land, W.Schramm, J.Stockbauer, V.Pierson, B.Boley)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, than is now available from the United States vital records. This objective will be accomplished by the following: 1) mailing or administering a questionnaire to mothers of VLBW infants, mothers of fetal deaths, and a sample of mothers of moderately LBW infants (1500-2499 grams) and normal birth weight infants (≥2500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; 2) conducting telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on the questionnaires; 3) developing procedures for abstracting information from hospital and physician records, including otherwise unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and 4) preparing an edited data tape for NICHD. In addition, mortality and results of follow-up evaluations will be available through the first year of life for this birth cohort.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02500-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
ETEC Seroepidemiology Evaluations in Alexandria, Egypt

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NAMRU-3 Unit, Cairo, Egypt (A.Mortagy, B.Kay, J.Murphy, M.Kleinosky); University of Alexandria (A.Mourad, M.Gaafar, M.Shasly); University of Goteborg (J.Holmgren, A-M.Svеeennerholm)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enterotoxigenic Escherichia coli (ETEC) diarrhea is hyperendemic among young Egyptian children, with an estimated cumulative incidence of 2.4 symptomatic ETEC episodes from birth to 36 months, and a balanced distribution of toxin phenotypes. These features make it logical to develop a field site for the evaluation of ETEC epidemiology and ETEC vaccines in Egypt. Recently, we have begun a collaborative project to develop a site in Abees (near Alexandria) for the study of pediatric diarrhea.

This project will follow a pediatric cohort with the following aims: 1) to determine the age-specific incidence rate of ETEC diarrhea from birth to 35 months, by toxin and colonization factor (CFA) phenotype; 2) to ascertain the strength of attribution of diarrheal symptoms to fecal isolation of ETEC; 3) to evaluate the protective relationship between titers of serum IgG antibodies to ETEC toxins and CFAs and the risk of diarrhea due to ETEC manifesting these virulence factors. Work on this project began in June, 1993; the work thus far has focused on protocol design, data form development, development of computer systems for data entry and management, and preparation of manuals of procedures. It is anticipated that the cohort study will, after conduct of a pretest, begin in November, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 02501-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Maternal Child Health Project of Assiut, Egypt

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.D. Clemens	Chief	EB, DESPR, NICHD
Other:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to investigate the incidence, determinants, and morbid sequelae of low birth weight in 20 rural villages in Assiut Governorate in Upper Egypt. The project includes the following major components: a complete census of the 20 villages to record demographic characteristics and environmental conditions of each household; weekly surveillance of births, deaths, and migrations to update the census; prospective monitoring of all deliveries with collection of data about the pregnancy and delivery and measurement of infant birth weights; and prospective weekly surveillance of all children in the villages aged 36 months or younger for symptoms and signs of diarrhea, acute respiratory infection, measles, malnutrition and growth disorders, or other diseases. Analyses will focus on evaluation of maternal, household, and obstetric characteristics as risk factors for low birth weight and on the relationships between birth weight, child morbidity, and growth patterns. The project will also include a service component, in which mothers, newborns, and children with specified morbid symptoms will be referred to community clinics which will be established in each community.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02502-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NICHD-Health Research Board of Ireland Neural Tube Defects Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Health Research Board of Ireland and Trinity College Ireland

LAB/BRANCH
Epidemiology Branch

SECTION
Pediatric Epidemiology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Epidemiology Branch (DESPR) is conducting a number of studies in collaboration with the Health Research Board and Trinity College, Ireland. These investigations are designed to determine the biochemical mechanisms by which folate reduces the risk for neural tube defects. Data and blood samples have been collected on a large proportion of Irish women delivering babies in Dublin. Samples from women whose pregnancy ended in the delivery of a child with a neural tube defect and control women whose pregnancy ended in the delivery of a normal child are being studied. Various aspects of folate metabolism and other nutritional measures are being examined. Ancillary studies are also being performed to determine whether other micro nutrients are involved in the pathogenesis of neural tube defects and how the risk for having an affected child varies from pregnancy to pregnancy in the same women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 02503-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
1991 Follow-up National Maternal and Infant Health Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.D. Overpeck Epidemiologist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
National Center for Health Statistics (M.D. Kogan and L.A. Fingerhut)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER:
0.2		0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The 1991 Longitudinal Followup to the National Maternal and Infant Health Survey (LF-NMIHS) of children born in 1988 was analyzed to assess the causes for medically-attended non-fatal injuries treated in all settings. The LF-NMIHS is a nationally representative survey based on birth and death records for the 1988 birth cohort and includes demographic and medical data on the child and family. The findings of the injury analysis were submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02504-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methodological Review: Low Birth Weight and Infectious Disease Mortality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three recent studies having reported an association between low birth weight and infectious disease mortality were evaluated with regard to six methodological standards for scientific validity. At most, only half of the standards were completely fulfilled in any study. Overall, the studies were particularly deficient with regard to description of methods of ascertainment of eligibility, achievement of nondifferential ascertainment of birth weight and of mortality, demonstration of nondifferential acquisition of diagnostic data regarding cause of death, and provision of explicit and unbiased cause of death classification. Our review of the published literature regarding low birth weight and infectious disease mortality demonstrates significant methodological limitations with all of these studies. This review also highlights the need for further research in this area in order to provide credible evidence regarding the presence and strength of a potential relationship between low birth weight and infectious disease mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02505-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Infectious Disease Mortality during Infancy: United States, 1987

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Staff Fellow	EB, DESPR, NICHD
Other:	J. Troendle	Staff Fellow	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is little information on risk factors for infectious disease mortality in infancy. Data from the NCHS 1987 Linked Birth/Infant Death data set is being analyzed in order to describe infectious disease mortality among U.S. infants as well as to identify potential determinants of infectious disease mortality during three periods: early neonatal, late neonatal, and postneonatal. The potential risk factors under study are derived from information available on birth certificates. Classification of cause of death is based on information listed on infants' death certificates. Hypotheses generated using this data set will be tested in the soon to be released NCHS 1988 and 1989 Linked Birth/Infant Death data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02506-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lack of Age-Appropriate Immunization among Infants Born in District of Columbia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Epidemiology Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	R. Brenner	Epidemiology Staff Fellow	EB, DESPR, NICHD
	B. Simons-Morton	Health Research Specialist	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The proposed program of research includes analytic studies (both observational and interventional) of immunization. The program will begin with a preliminary phase including needs assessment and feasibility studies. The findings from this phase will be used in the planning and execution of a cross-sectional and a cohort study, as well as an intervention study. The observational and intervention studies are seen as collaborative efforts to develop a better understanding of how immunization can be promoted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02507-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prostaglandin Excretion in Preeclampsia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J.L. Mills	Chief, PES	EB, DESPR, NICHD
	R.J. Levine	Expert	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A disturbance of prostaglandin metabolism is currently believed to be one of the major mechanisms in the pathogenesis of preeclampsia. Most previous studies of this theory, however, have included only women who had already developed clinically overt disease. In order to investigate whether prostaglandin abnormalities precede the onset of preeclampsia, we have designed a nested case-control study using stored urine specimens prospectively collected as early as thirteen weeks gestation from women participating in the NICHD Trial of Calcium to Prevent Preeclampsia (CPEP). In this study, we will compare the urinary excretion of prostaglandin and thromboxane in preeclampsic women before the diagnosis of preeclampsia to that in normal pregnant women at comparable gestational ages. Because half of the women in the CPEP trial will be taking two grams of oral calcium daily, we will also be able to examine the effect of oral calcium supplementation on the excretion of these prostaglandin metabolites.

050

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02508-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Surrogate Measurements for Birth Weight and Gestational Age

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	Nebiat Tafari	Visiting Scientist	EB, DESPR, NICHD
	James Troendle	Staff Fellow	BMSB, DESPR, NICHD
	John D. Clemens	Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Low birth weight and prematurity are important sources of neonatal mortality and morbidity worldwide. In developing countries, it is often difficult to measure birth weight and gestational duration directly, because most deliveries are not medically attended. Therefore, a number of studies have examined the utility of using neonatal anthropometric measurements as surrogate screening tests to identify low birth weight and preterm infants. The methodology employed in these studies, however, has not met the standards which have been recommended for the development of screening tests or medical decision rules. Our analysis will illustrate these standards using data from Ethiopia to develop an algorithm to enable practitioners in outpatient settings to use neonatal measurements to identify low birth weight, preterm infants needing referral for special care.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02509-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Comparison of Methods of Provision of Warmth to Low Birth Weight Newborns

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	M.R. Rao	Visiting Associate	EB, DESPR, NICHD
	K.F. Yu	Math Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS (if any)
Department of Pediatrics and Child Health, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia (N. Tafari and C. Green-Abate)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS.	PROFESSIONAL:	OTHER.
0.55	0.55	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A randomized clinical trial comparing two alternate methods of provision of warmth to low birth weight (LBW) newborns was undertaken in a referral hospital in Addis Ababa, Ethiopia. The standard, convectively heated infant incubator (INC) is the accepted method of thermal protection of the LBW and sick newborns. Because INC care requires high technology, the acquisition and implementation of this method is beyond the resources of many developing countries. Until recently the only alternative method of provision of warmth to LBW newborns has been the space heated room (SHR) to an average temperature of 30°C. The SHR has been an unsatisfactory alternative because the climatic conditions cannot be individualized to meet the thermal needs of LBW newborns of varying sizes and maturity, and the high environmental temperature required is too uncomfortable for care providers. In response a thermostatically heated, water-filled mattress (HWM) was developed. Since newborns cared for on the HWM are covered, there is less of a need to regulate the thermal environment within a narrow range as naked newborns in INCs. On the other hand, unlike the situation in SHR, the HWM provides a thermal environment that can be adjusted to suit the individual thermal needs of the newborn.

Analysis of the results of the randomized trial showed that care in the SHR produced clinically significant cold stress and significant risk of hyperthermia and hypothermia from overheating or inadequate heating. The HWM, on the other hand, was not associated with clinically significant cold stress or risk of inadequate heating or overheating and thus may constitute a feasible and clinically acceptable alternative in providing warmth to LBW newborns during the neonatal period.

09

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 02510-01 EB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Diet, Maternal Nutritional Status, Blood Pressure and Fetal Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	H.W. Berendes	Director	DESPR, NICHD

COOPERATING UNITS (if any)
Ethiopian Nutrition Institute (Z.W. Gabriel, E. Woohib)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.45	0.45	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study was sponsored by the City Council of Addis Ababa as part of health and nutritional assessment of low income families prior to planning a food supplementation program for women and children. This particular program was initiated because of sudden increase in the price of essential food items following implementation of government policies that preferred state control of production and distribution of all essential commodities.

The study of the effect of home diet on fetal growth arose from the observation that in Ethiopia, where severe deficiency in food energy is prevalent, there appears to be no corresponding deficit in fetal growth. This observation is at variance with the generally held view that dietary energy deficit during pregnancy leads to fetal growth retardation. However, several lines of recent evidence suggest that fetal growth is protected through adaptive processes. Among possible adaptive mechanisms area: 1) higher physical work efficiency; 2) greater mobilization of maternal energy reserves (fat deposits); and 3) hemodynamic adjustments to maintain adequate uterine perfusion.

Data analysis to date showed that the dietary energy deficits in low income families were associated with deficiencies in nutritional status of the gravida during the third trimester and the puerperium as reflected by reduced skinfold thickness and body mass index. Third trimester diastolic blood pressure was significantly reduced in poorly nourished gravida. Although a statistically significant difference of 180 g in birth weight was detected between the offspring of the undernourished and adequately nourished, it is not yet clear whether this difference is due to diet alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES – PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00131-19 HGB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Human Biochemical Genetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI:	William A. Gahl	Head	HGB, NICHD
Others:	Isa M. Bernardini	Chemist	HGB, NICHD
	Stephen G. Kaler	Medical Staff Fellow	HGB, NICHD
	Donna M. Krasnewich	Medical Staff Fellow	HGB, NICHD
	Si Houn Hahn	Medical Staff Fellow	HGB, NICHD
	Linda K. Gallo	Medical Staff Fellow	HGB, NICHD
	Gordon Holt	Senior Staff Fellow	HGB, NICHD
	Geraldine McDowell	IRTA Fellow	HGB, NICHD

COOPERATING UNITS (if any)
See Attached

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Human Biochemical Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Members of the Section continue to care for over 100 patients with nephropathic cystinosis. Pre-transplant patients receive oral phosphocysteamine, which has allowed for maintenance or growth of renal function in these individuals. Six pre-transplant patients were also treated with oral L-carnitine which restored their plasma levels to normal and resulted in normal muscle carnitine levels after approximately 5 years of therapy. Post-transplant patients are studied for their nonrenal complications and are offered oral phosphocysteamine. Cysteamine eyedrops (0.5%) have dissolved corneal crystals in young children and removed the haziness from the eyes of older children. A gene exclusion map has been constructed for cystinosis using CA repeats as polymorphisms in linkage analysis.
2. Seventeen patients with Menkes disease have received daily subcutaneous injections of copper histidine. There has been modest clinical improvement in several patients. Plasma and cerebrospinal fluid neurotransmitter ratios have proven useful for the diagnosis of this disorder. Specific molecular mutations are being pursued in individual patients.
3. Metal regulatory factors, which bind to the metal regulatory elements of the human metallothionein IIA gene, are being studied using mobility shift assays and protein blotting. A 34 kd metal regulatory factor is being pursued in some depth.
4. Investigations continue into Carbohydrate Deficient Glycoprotein Syndrome. The carbohydrate pattern of one serum glycoprotein, α-1 antitrypsin, is abnormal in its ratio of N-acetylglucosamine to mannose and of galactose to mannose. This provides a clue into the basic defect in this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD00137-19 HGB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation and Expression of the UDP-Glucuronosyltransferase Gene Family

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ida S. Owens	Head	HGB, NICHD
Others:	Joseph Ritter	Senior Staff Fellow	HGB, NICHD
	Marco Ciotti	Visiting Fellow	HGB, NICHD
	Aprile Pilon-Clayton	IRTA/Staff Fellow	HGB, NICHD
	Michelle Nguyen	Biological Aid	HGB, NICHD

COOPERATING UNITS (if any)

Frederick Cancer Research Facility, Frederick, MD (Ronald Lubet).

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Genetic Disorders of Drug Metabolism

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
4.6	3.5	1.1

CHECK APPROPRIATE BOXES.

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our description of the *UGT1* locus has proven critical for the determination of genetic defects in Crigler-Najjar Type 1 (CN-1) patients. The complex (*UGT1A - UGT1G*) codes for at least 2 bilirubin (Br), 3 Br-like, and 2 phenol transferases. Seven different exon 1s, each with an upstream promoter and each encoding the amino terminus of an isoform, are arrayed in series with 4 common exons (encoding seven identical carboxyl termini) in the 3' region of the locus. Predictably, a critical mutation in a common exon inactivates the locus; a deleterious mutation in an exon 1 as we describe for the *UGT1A* gene in two CN-1 patients affects the amino terminus of that single isoform. The code in patient 1 for the predominant Br isozyme, the HUG-Br1 protein, is missing codon 170 (phe) in exon 1 of *UGT1A* abolishing a diphenylalanine in a conserved hydrophobic region A encoded by codons 161-180. Studies with this mutant led to the discovery of the previously unknown major pH optimum (6.4) in contrast to the routinely used pH (7.6) which we show is only 1/3 as effective for Br glucuronidation using either HUG-Br1 protein or human liver microsomes. HUG-Br1-170, inactive at pH 6.4, has normal activity at pH 7.6, a result consistent with a pH-sensitive mutant. The HUG-Br1 protein and microsomes glucuronidate Br best at low ionic strength. The wild-type protein K_m value for Br is 2.5 μM (pH 6.4 or 7.6); that for HUG-Br1-170 is 5.0 μM (pH 7.6). In the genome of CN-1 patient 2, a point mutation (G->C, gly to arg) at codon 276 abolishes a diglycine in a conserved region B encoded by codons 270-288 in exon 1 of *UGT1A*. The HUG-Br1-276, inactive at pH 6.4 and 7.6, defines micro-structure/region B which has 7 hydrophobic-, 7 amide-containing-, and 4 α-helix-breaking amino acid residues. Point mutations in common exons 2 and 4 of CN-I patients 3 and 4 with G->A (codon 309) and C->T (codon 376) uncovered conserved region C encoded by codons 298-315 and conserved residue D, ser/ala, respectively. In the common carboxyl terminus micro-regions C and D presumably interact with the common substrate, UDP-glucuronic acid, while hydrophobic regions A and B most likely interact with the lipophile-like Br substrate. Characterization of human transferase encoded by the UDPGTh-3 cDNA, establishes that it specifically glucuronidates the dihydrotestosterone and androstane diol steroids; its mRNA is located in testis and prostate, as well as liver, suggesting a critical role for udpgth-3 in target tissue. The absence of its mRNA, and the presence of that for four other isoforms in the liver of a patient with benign prostate hyperplasia (characterized by depressed glucuronidation of dihydrotestosterone) is supportive evidence for its biochemical importance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00404-11 HGB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jean DeBrohun Butler	Senior Investigator	HGB, NICHD
Others: Michelle Evans	Biology Aid	HGB, NICHD

COOPERATING UNITS (if any)

Cooperating Units: Laboratory of Developmental and Metabolic Neurology, NINCDS (Peter G. Pentchev); Laboratory of Cell Biology and Genetics, NIDDK (Mark Levine); Section on Developmental Genetics, Human Genetics Branch, NICHD (Anil B. Mukherjee); Laboratory of Cellular and Developmental Biology, NIDDK (Joan Blanchette-Mackie).

LAB/BRANCH

Human Genetics Branch

SECTION

Unit on Genetic Diseases Involving Cystine Storage

INSTITUTE AND LOCATION

NICHD, NIH, 10/10N308 Bethesda, MD 20892.

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Commercially obtained cystine binding protein of E. coli is heterogeneous. Purification gave four proteins. One protein bound cystine and was not similar to known proteins in data banks. Another protein was highly similar to a known histidine binding protein of S. typhimurium and can be used to specifically analyze tissues for histidine content as is now done for cystine.

2. Normal fibroblasts can reversibly be induced by hydrophobic amines and certain steroids to mimic NPC disease. With this model we are studying the development of this disease and subsequent restoration to normal using known agonists and antagonists of intracellular lipid transport and metabolism. We find that trafficking of intracellular cholesterol may be via the golgi using both biochemical and cytochemical methods.

3. Normal and cystinotic fibroblasts have two ascorbic acid transporters, a high affinity Na+ dependent transporter and a low affinity Na+ independent transporter, neither of which is due to diffusion. The transporters are not different in the two types of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00408-10 HGB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heritable Disorders of Connective Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Joan C. Marini	Senior Investigator	HGB, NICHD
Others:	Jane D. Filie	NRSA Fellow	HGB, NICHD
	Qin Wang	Fogarty Fellow	HGB, NICHD
	Adriana Sarafova	Fogarty Fellow	HGB, NICHD
	Laura Russell	Medical Staff Fellow	HGB, NICHD
	Bonnie Orrison	Biologist	HGB, NICHD

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Cc (N.L. Gerber); Laboratory of Developmental Endocrinology (G. Chrousos)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Connective Tissue Disorders

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section has continued its studies aimed at elucidating the molecular mechanisms of heritable disorders of connective tissue, specifically osteogenesis imperfecta (OI) and Ehlers-Danlos (EDS), and at applying this information to treatment of these disorders.

One primary interest of the Section is to identify the collagen mutations in patients with OI and EDS and determine the relation-ship between the type and location of the mutation and the severity of the connective tissue disorder. Mutations in the $\alpha 2(I)$ collagen chain identified by this Section and other labs have provided additional support for the regional model we have proposed.

A second primary interest of the Section is to develop selective antisense suppression of the mutant collagen allele as an approach for therapeutic intervention. We have used thio oligos targeted to mutant mRNA and nuclear RNA in the cultured fibroblasts of a patient with type IV OI. We have achieved selective suppression of the mutant allele and are pursuing approaches such as ribozymes and vector constructs to increase suppression efficiency.

A third focus of interest which we have been developing is in the bone biology of OI. We are using cultured osteoblasts to study the way bone cells modify and secrete mutant collagen. We are also pursing the secondary non-collagenous abnormalities of OI matrix.

In clinical studies, we are continuing our treatment trial of growth hormone in short children with OI to determine its effects on growth stimulation, bone density and bone morphometric properties. We are continuing our collaborative interests in the neurological aspects of OI and in maximizing the physical functioning of OI children though aggressive rehabilitation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00410-07 HGB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism in Children with Glycogen Storage Disease, Type I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James b. Sidbury	Scientist Emeritus	HGB,NICHD

COOPERATING UNITS *(if any)*

Laboratory of Theoretical and Physical Biology, NICHD (N. Esteban and A.L. Yergey)

LAB/BRANCH
Human Genetics Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
  - ☒ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TRANSFER TO LTPB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD00414-04 HGB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neurogenetics and Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Lawrence Charnas, M.D., Ph.D.	Senior Staff Fellow	HGB, NICHD
Others:	Christine Potter	Biologist	HGB, NICHD
	Steven Scott	Junior Fellow	HGB, NICHD
	Dalano Hoang	Stay in School	HGB, NICHD

COOPERATING UNITS *(if any)*
Department of Rehabilitation Medicine, CC (U. A. Chaudhry); OD and MNB, NINDS (M. Dalakas, C. Luciano) Clinical Branch, NEI (M. Kaiser-Kupfer), HHMI, University of Pennsylvania (R.L. Nussbaum), National Naval Medical Center (R.C. Miller), Johns Hopkins Medical Institutions (T. A. Brushart), CPB, NIMH (H. Leonard, A. J. Allen),

LAB/BRANCH
Human Genetics Branch

SECTION
Human Biochemical Genetics

INSTITUTE AND LOCATION
NICHD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical Studies:

a) Nonsense mutations in OCRL-1 have been identified and help confirm OCRL-1 as the gene defect in Lowe Syndrome. The specific mutation does not predict the severity of disease. Stereotypy (random, repetitive, purposeless movement) is a cardinal feature of the behavioral disturbance in Lowe Syndrome and may be controllable with medication. Prenatal detection of cataracts may not be a reliable diagnostic test for Lowe syndrome. b) The myopathy of cystinosis is quite focal, is arrested by cysteamine therapy, and is amenable to surgical treatment with marked improvement in function. c) Newly recognized neurologic manifestations of osteogenesis imperfecta include abnormal kinetics of head circumference growth. Symptomatic basilar invagination may limit improvement in ambulation and attempts at growth stimulation.

Laboratory Studies:

a) A brain-specific exon due to alternative splicing of OCRL-1 has been identified in mouse and man. Different patterns of splicing may also be present in other neural precursors. b) cDNA for the mouse homolog (MUSINOS) of human inositol polyphosphate-5-phosphatase (HUMINOS) has been identified and errors in published sequence identified in critical homology regions. c) The expression of OCRL-1 in fetal mouse is predominantly in the nervous sytem, adrenal medulla, and kidney, with lower expression in connective tissue stroma; MUSINOS is expressed more diffusely and at lower levels. d) The gene for HUMINOS has been mapped to 1p34.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00415-03

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology and molecular Biology of Arachidonate Metabolism & CFTR Mutation in CF.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

Anil B. Mukherjee, Ph.D., M.D. P.I.
Lucio Miele, M.D., Ph.D. Visiting Associate Co-P.I.
Eleonora Cordella-Miele, M.D. Visiting Associate

COOPERATING UNITS (if any)

University of Alabama (R. Frizzell).

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development,NIH

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ *(c) Neither*

☐ *(a1) Minors*

☐ *(a2) Interviews*

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is established that mutation of the gene coding for a cAMP-regulated anion channel, the cystic fibrosis transmembrane regulator (CFTR) protein causes Cystic Fibrosis (CF). The deletion, Phe 508 (ΔF^{508}), in CFTR is the most common mutation in CF. A major complication of CF is extensive and destructive inflammation of the airways. The secondary biochemical and functional alterations caused in CF epithelial cells by a defect in CFTR function are not yet completely understood. Recent evidence suggests that cells carrying the ΔF_{508} mutation in the homozygote state have altered acidification of intracellular organelles and defective c-AMP-induced membrane recycling. It has been suggested that the regulation of phospholipase A_2 (PLA_2)-catalyzed arachidonic acid release may be defective in CF lymphocytes and fibroblasts. Last year we have described the results of Calcium ionophore A23187-induced arachidonic acid release in the following cell lines: T84 cells (a colon carcinoma line which expresses high level of normal CFTR; CFPAC-1 cells (a pancreatic carcinoma line from a delta F_{508} CF patient) and clones derived from CFPAC-1 cells after transfection with a retroviral vector containing the normal CFTR gene (CFPAC-PLJ-CFTR) or with vector only (CFPAC-PLJ). Our data indicated that those lines containing a mutant CFTR and showing defective c-AMP-induced Cl^- efflux have a 5-10 fold higher arachidonate release than that in the controls. This increased release is dependent upon extracellular Ca^{++} and is further enhanced by phorbol-12-myristate, 13-acetate(PMA, stimulator of PKC) but not by the inactive derivative 4α phorbol,12,13 didecanoae and is inhibited by staurosporine (a PKC inhibitor). An inhibitor of PLA_2, quinacrine (200μM) abolishes arachidonate release in these cells. These data suggested that Ca^{++} and pKC-stimulated arachidonate release, possibly catalyzed by PLA_2, is disregulated in cells carrying the delta F_{508} CFTR mutation in CF. These data indicated that an alteration of a Ca^{++}, PKC-regulated arachidonate release raising the possibility that this abnormality may be due to activation of $cPLA_2$. We now report that although the basal activity of $cPLA_2$ is equivalent in the control as well as in CF cells there may be agonist induced enhancement of activity of this enzyme in CF cells. Should this be the case $cPLA_2$ inhibition may regulate the abnormal arachidonate release and thereby controlling the severe inflammation commonly found in the respiratory tracts of CF patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

717-03

Z01 HD 00~~136-02~~ HGB

PERIOD COVERED

October 1, 1992-September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis of α1-Antitrypsin Deficiency

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark Brantly	Senior Staff Fellow	HGB, NICHD
Others:	Victor Laubach	Pre-IRTA	HGB, NICHD
	Carol Blank	Medical Staff Fellow	HGB, NICHD
	Jeff Hildesheim	Pre-IRTA	HGB, NICHD
	Jeffrey Redwine	Biologist	HGB, NICHD

COOPERATING UNITS (if any)

See Attached

LAB/BRANCH

Human Genetics Branch

SECTION

Unit on Genetic Disorders of Secreted Proteins

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
3.6	2	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies have focused on determining the molecular basis of α1-antitrypsin (α1AT) deficiency variants utilizing natural mutations of the α1AT gene to assess structure-function relationships of α1AT and to explore regulatory mechanism of α1AT gene expression. In the past year the lab has identified an intronic sequence that enhances α1AT gene expression, characterized the molecular basis of the first splicing mutation in the α1AT gene, developed a model for understanding the molecular basis of heterogeneity among α1AT variants, begun the characterization of a group of frameshift mutations that terminate in the same structural region of the α1AT protein, determined the relationship of α1AT PI type to viral hepatitis. Furthermore the lab completed the kindred analysis and developed a screening assay for the novel null allele QOtrastervere. Finally, the α1AT phenotyping lab completed the intial identification of 1161 α1AT deficient individuals as part of the α1AT Deficiency Registry Study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES – PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00910-14

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Uteroglobin, cc10kDa protein, Phospholipase A_2, Osteopontin and HIV-1 Protease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

Anil B. Mukherjee, M.D.,Ph.D.	P.I.
Lucio Miele, M.D.,Ph.D.	Visiting Associate
Eleonora Cordella-Miele, M.D.	Visiting Associate
Giuditta Mantile, Ph.D.	Visiting Fellow
Alessandro Peri, M.D.	Visiting Fellow
Ijeoma Nwaeze	Stay-in-School

COOPERATING UNITS (if any)
University of Naples, Italy (***N. Barbato***); University of Rome(***S.Beninati***); Georgetown University (***R.Dhanireddy***); George Washington University (***M.Manyak***); McGill University(***B.B.Mukherjee***); Harvard Medical School (***D.R.Senger***); University of Pittsburgh (***G.Singh, S.Katyal***), Naval Research

LAB/BRANCH
Human Genetics

SECTION
Developmental Genetics

INSTITUTE AND LOCATION
National Institute of Child Health & Human Development, NIH

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have:(a) established and characterized two endometrial epithelial cell lines which are temperature-sensitive for differentiation and express uteroglobin gene in response to steroid hormones;(b) confirmed our preliminary observations that phospholipase A_2 (PLA_2) is activated by transglutaminase (TG)-mediated polyamination of this enzyme;(c) identified a specific region of group I PLA_2(residues 21-40) interaction of which with a neutralizing antibody leads to complete inhibition of its activity; (d) obtained overexpression of recombinant human uteroglobin (hUG) in E.coli, and demonstrated that this recombinant protein is a potent inhibitor of PLA_2 activity and an excellent substrate of TG. (e) performed site-directed mutagenesis of hUG and expression of recombinant mutated protein. Preliminary results suggest that Lys 43 of hUG is critical for its PLA_2 inhibitory activity; (f) established that hUG gene is expressed in various tissues and cells other than the pulmonary alveolar Clara cells as was originally thought; these results demonstrate the similarity between the rabbit and hUG expression; (g) confirmed the preliminary results that surfactant-producing rabbit pulmonary alveolar type II cells synthesize and secrete UG, thereby, protecting the phospholipid component of the surfactant in the lung; (h) fully characterized a region of osteopontin (OP) which specifically binds Ca^{++}; (i) confirmed the preliminary results that in the milk of mothers who delivered premature infants, the OP level is significantly higher than that in the milk of mothers who delivered at term, suggesting a possible role of OP in Ca^{++} transport from mother to the neonate; (j) established that OP-fibronectin interaction in bone and extracellular matrix (ECM) protein is mediated by transglutaminase (TG); (k) discovered a novel splicing of OP mRNA with possible regulatory role in transcriptional and/or posttranscriptional events, possibly explaining the multifunctional role of OP in diverse cell types and tissues; (l) confirmed the preliminary observation that a TG-mediated polyamination of HIV-1 protease, similar to the ones reported for PLA_2, which may have implication for its activation; (m) developed a rapid, inexpensive, non-radioactive and simple quantitative PCR-ELISA for quantitation of gene expression and detection of mutation. This assay is potentially applicable to prenatal genetic diagnosis and the diagnosis of viral (including HIV), pro- and eukaryotic infections; international and US patent applications have been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 000912-14

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Regulation and Cellular Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Janice Y. Chou	Head	HGB, NICHD
Others:	Kei-Jian Lei	Visiting Scientist	HGB, NICHD
	Naohiro Wada	Visiting Fellow	HGB, NICHD
	Leslie Shelly	Visiting Fellow	HGB, NICHD
	Margaret Chamberlin	NRC Biotechnology Fellow	HGB, NICHD
	Chaehwa Park	Visiting Fellow	HGB, NICHD See Attached

COOPERATING UNITS (if any)

Institute of Cell and Tumor Biology, German Cancer Research Center, Federal Republic of Germany (Drs. G Schutz and S Ruppert); University of Florida (Dr. C-L Chen); University of Colorado Health Sciences Center, CO (Dr. KK Leslie); Georgetown University (Dr. Y-W Chan)

LAB/BRANCH

Human Genetics Branch

SECTION

Cellular Differentiation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
9.5	7	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have characterized cDNAs and genes encoding human and mouse glucose-6-phosphatase (G6Pase), the enzyme deficient in glycogen storage disease (GSD) type Ia. This autosomal recessive disorder is characterized by hypoglycemia, hepatomegaly, growth retardation, lacticacidemia, hyperuricemia, renal dysfunction and hepatic adenomas. Moreover, we have identified several mutations in the G6Pase gene of affected individuals and confirmed that these alterations completely inactivate the enzyme. These results unequivocally establish the molecular basis of GSD type Ia and open the way for future gene therapy.

We have characterized cDNA and the gene for mouse liver S-adenosylmethionine (AdoMet) synthetase which catalyzes the synthesis of AdoMet functioning in transmethylation and trans-sulphuration. We also demonstrated that the -365 to -2 DNA region upstream of the transcription start site of the AdoMet synthetase gene contains promoter elements and the -518 to -366 DNA region involves negative regulation.

We have shown that pregnancy-specific glycoproteins (PSGs) containing short C-domains are secretory proteins, except PSG11w which contains an 81-residue C-domain and is largely retained in cells. By studying the synthesis and processing of PSG, PSG chimeras, and PSG11w deletion mutants in the absence or presence of Brefeldin A, a drug that impedes protein transport from ER to the Golgi system, we have demonstrated that the PSG C-domains specify protein secretion and stability.

We have shown that the -835 to -34 DNA region upstream of the translation start site of the PSG12 gene contains both positive and negative elements that control PSG expression and the -172 to -34 DNA region functions as a promoter. The PSG12(-172/-34) DNA forms four complexes with protein factors in human placental cell extract. Mutagenesis studies have located the core enhancers at nucleotides -148 to -141 and -60 to -55 of the PSG12 promoter.

We have demonstrated that the -156 to -1 DNA region upstream of the transcription start site of the germ-cell alkaline phosphatase (GCAP) gene, which contains three GC-rich nuclear protein binding sites (I, II, and III), directs GCAP expression in choriocarcinoma cells. Site-directed mutagenesis that prevents binding of nuclear proteins to sites I and/or II, results in the loss of factor binding and reduced activator activity. The GCAP promoter that contains an intact binding site III but altered sites I and II has little activator activity, suggesting that protein-protein interaction is important for GCAP gene activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00054-19 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Behavior Analysis of Vocal Communication in Squirrel Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S, J. Suomi	Head, SCBG	LCE, NICHD
Other:	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

M. Biben

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Unit on Brain, Behavior, and Communication

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Unit's continuing focus on the vocal behavior of nonhuman primates this year concentrated on early vocal development, and the use of vocal interaction in social groups of adults. Infant vocalizing (in the first year of life) varies greatly from that of adults. Calling is more frequent and the mix of calls used is very different. Most important are the gradual structural changes evident in at least one vocalization, the "chuck" call, which we have previously identified as being an affiliative call with many language-like properties. Plasticity in vocal production is not typical of nonhuman primate vocal ontogeny, as it is in humans, and structural changes in the chuck call may be the first evidence of a role for experience or learning in a monkey call. Additional evidence of plasticity comes from our latest study of chuck usage in adult squirrel monkeys. Using a group of adult females, we broadcast to them the recorded calls of younger adult females who were born into the group but who had been removed from it before their distinctive chuck calls had matured. The older animals showed recognition of the younger females' chucks despite never having heard them before. Structural similarities between the chucks of the older and younger females provide a basis for recognition, but the origin of these (or other, undetected) commonalities is unclear. Daughters' chucks did not resem 'e mothers' more than others', and mothers were not more responsive to their own daughters. This suggests tnat common properties in the chuck calls of animals sharing genes or rearing environment ("familiarity" that is critical in eliciting a response from group members) may have a learned component. Chucks are extremely versatile calls, having both individually distinct, and group-identifying characteristics, in addition to flexible usage based on context. Certainly, the developmental life history of squirrel monkeys provides the opportunity for learning, as youngsters are exposed to millions of chuck calls from mothers and allomothers. There is no doubt that learning, if it figures at all in the production of nonhuman primate calls, has nowhere near its importance in human development. Nonetheless, by identifying points where learning occurs, we may gain insight into the kinds of activities where learned elements may have been most beneficial, and appeared earliest, in our ancestors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01106-10 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Continuity of Individual Differences in Reactivity in Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. J. Suomi	Head	LCE, NICHD
K. L. Rasmussen	Senior Staff Fellow	LCE, NICHD
M. Champoux	Staff Fellow	LCE, NICHD
J. D. Higley	Staff Fellow	LCS, NIAAA
G. D. Byrne	IRTA Fellow	LCE, NICHD
D. Smucny	Pre-IRTA Fellow	LCE, NICHD
S. Higley	Research Technician	LCE, NICHD

COOPERATING UNITS *(if any)*

NIAAA (Linnoila, Goldman; NIMH (Doudet); Delta Reg. Primate Ctr. (Clarke); Primate Lab., U of Wisconsin (Schneider); Istituto di Psicologia, CNR (Visalberghi); U or Puerto Rico (Phoebus); U of Colorado (Landenslager); SUNY - Buffalo (Berman)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.1	3.5	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates primate biobehavioral development through comparative longitudinal studies of rhesus and capuchin monkeys, with special emphasis on characterizing individual patterns of differential behavioral and physiological responses to environmental novelty and challenge and on determining long-term developmental consequences for individuals of different genetic backgrounds reared in different physical and social environments. During the past year striking differences between rhesus monkey infants whose parents came from wild populations in China and those whose parents were of Northern Indian heritage were found for measures of neonatal reflexes, activity patterns, ability to sustain attention, and temperament during the first month of life. Because both the Chinese derived and Indian derived infants were reared under identical nursery conditions, these differences could not be attributed to differential postnatal experiences. Distinctive patterns of heritability for serotonin, norepinephrine, and dopamine metabolites were found in a large sample of laboratory-born rhesus monkeys reared in comparable environments. Long-term behavioral and physiological effects of differential early rearing experiences were also documented in these monkeys during adolescence and early adulthood. The specific relationships between behavioral expressions of fear and impulsive aggression and characteristic patterns of adrenocortical reactivity and serotonin metabolism previously disclosed for captive rhesus monkeys under laboratory conditions were basically replicated in a wild population of free-ranging rhesus monkeys. Finally, initial studies of the effects of short-term separation in capuchin monkey subjects revealed a range of individual differences in biobehavioral reactions comparable to that observed in rhesus monkeys, despite major differences in fundamental aspects of the mother-infant relationship between these two monkey species.

006

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01107-10 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adaptation of Laboratory Reared Monkeys to Field Environments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. J. Suomi	Head	LCE, NICHD
A. Timme	Fogarty Fellow	LCE, NICHD
P. O. Wagner	Research Psychologist	LCE, NICHD
C. Price	Biologist	LCE, NICHD
M. Jorgensen	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

OACU (Bayne); Dept. of Psychology, U. of Massachusetts (Novak); Dept. of Psychology, Georgia State U. (Hopkins); U. of Florida (Boinski)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.1	2.5	2.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates how rhesus monkeys and other nonprimate species born and raised under different laboratory conditions adapt to placement into naturalistic outdoor environments that contain specific physical and social features of the monkeys' natural habitat. Adaptation is assessed by examining behavioral repertoires and by monitoring a variety of physiological systems in these subjects, yielding broad-based indices of relative physical and psychological well-being. The responses of subjects to experimental manipulations of selected features of their respective environments are also assessed in similar fashion. During FY93 a new shelter facility for the LCE's 5-acre outdoor enclosure at the NIHAC was completed and occupied by the resident rhesus monkey troop, greatly expanding the opportunities for carrying out experimental manipulations and physiological sampling in these free-ranging monkeys. Initial data collection focused on documenting the differential behavioral and physiological reactions of individual troop members to this major change in their physical habitat. A second study investigating spatial memory capabilities regarding the location of familiar and novel objects within the 5-acre enclosure was begun, while a third study compared the nature and rate of triadic interactions (involving mothers, infants, and other adult females) observed in this free-ranging troop with those seen in wild troops of rhesus monkeys on Cayo Santiago, PR. Pioneering studies of complex tool use and its relationship to laterality and object manipulation propensities in capuchin monkeys were completed in FY93, and a new experimental paradigm for evaluating complex cognitive and perceptual-motor capabilities that utilizes video game technology was developed and standardized for this monkey species.

012

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01108-09 LCE

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparative Studies of Play Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S. J. Suomi	Head, SCBG	LCE, NICHD
Other:	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

M. Biben

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Unit on Brain, Behavior, and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our current emphasis in the study of play is investigation of the motivational or social conditions promoting the appearance of playful behavior in the squirrel monkey. Developmental and functional analyses of human play have been the subject of extensive clinical interest, particularly as a symptom and early predictor of psychopathology. Comparable studies of play in animals have been few, although it is commonly assumed that play in animals is an indicator of good mental as well as physical health. Play behavior is ubiquitous in young mammals and is usually the chief means of social interaction between youngsters. Nonetheless, play appears to be quite sensitive to stressors, dropping out of the repertoire, or being greatly curtailed, in response to nutritional or environmental stress, and in some cases to emotional stress, e.g. loss of the mother. In captive animals, particularly juveniles, playfulness is thought to be a sign of general well-being. The present experiments are designed to investigate a possible relationship between a physiological measure of stress (plasma cortisol level) and a behavioral assessment of play, using as subjects a population of young squirrel monkeys, genus *Saimiri*. Juvenile squirrel monkeys exhibit an extensive repertoire of play behaviors and, independently, their physiological and endocrine responses have been well studied, providing a particularly favorable opportunity to correlate physiology and behavior. The first hypothesis being investigated is that an inverse relationship exists between an individual monkey's plasma cortisol level (a measure of stress) and his or h level of play. Our results so far indicate that the predicted negative relationship is true; plasma cortisol and amount of time spent in play (measured on a rating scale) showed a significant negative correlation both for the baseline condition and over all conditions in the study. The experimental paradigm attempted to manipulate stress by altering an individual's social environment; re-pairing animals with new partners increased their cortisol levels reliably, particularly for animals with low baseline levels. Play was also disrupted by re-pairing, as expected. Establishing a correlation between stress and play (essentially, providing a sound basis for the conventional wisdom that holds that they are incompatible) has practical as well as theoretical significance. As a result of recent and pending legislation, investigators working with captive primates must assure the well-being of the animals in their care. Yet few assessment measures exist. Measuring (or simply observing) play may be an expedient, non-invasive and supportable indicator of psychological well-being for young animals, particularly useful in light of new mandates requiring pair housing of primates wherever possible.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01111-09

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Affecting Nurturant Behavior Towards Infant

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. J. Suomi	Chief	LCE, NICHD
G. Virgo	NRSA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
NIMH (Huffman, del Carmen); U. of Maryland (Porges); Brown U. (Lester); U. of Delaware (Pedersen); Purdue U. (Bryan)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Unit on Parent and Infant Studies

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

023

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01112-06 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Home and Out-of-Home Care on Child Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	H. Weßels	Visiting Fellow	LCE, NICHD
	K.J. Sternberg	Research Psych	LCE, NICHD
	S. Chace	Research Psych	LCE, NICHD

COOPERATING UNITS *(if any)*

Center for Human Growth and Development, U of Michigan (Bookstein); Institute of Psychology, University of Goteborg (Broberg, Hwang).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves a longitudinal study conducted in Sweden examining the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age. Multivariate analyses have consistently indicated that type of care has no reliable impact on child development, but that the quality of home care and the quality of alternative care have substantial effects on the children's verbal abilities, their social skills and their behavioural attitudes. The children were most recently assessed when they averaged 8 years of age and were completing their first year of formal schooling. To date, data on the development of verbal abilities as well as the development of personal maturity (as measured by field-independence, ego-control, and ego-resilience) have been analyzed in relation to the varying child-care histories the children have experienced since their first enrollment in the study. These analyses indicate that family daycare has modest but significant effects on both achievement and personal maturity, whereas center-based care has beneficial effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01113-07 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	S. Chace	Research Psych.	LCE, NICHD

COOPERATING UNITS *(if any)*

Dept. of Family Studies, Brigham Young University (Barber); Dept. of Psychology, Univ. of Maryland, Baltimore County (White); Xiaohong Xu.

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project's goals were to describe the psychosocial context of adolescent sexual and nonsexual risk-taking and problem behaviors, especially as they relate to a high risk of contracting AIDS and other STD's, and to explore the long-term effects of early parenthood on families. In a series of studies, we have shown that involvement in different types of problem behaviors tends to co-occur. A book summarizing research on this topic is now in press, and no additional research in this area is planned.

028

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01114-06 LCE

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Individual Differences in Physical and Affective Functioning in Infancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	M. P. Fracasso	NRC Fellow	LCE, NICHD
	B. Leyendecker	Fogarty Fellow	LCE, NICHD
	A. Scholmerich	Fogarty Fellow	LCE, NICHD
	M. T. Estrada	Pre-IRTA Fellow	LCE, NICHD
	L. Shelley	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
Department of Psychology, U of Maryland (S. Porges); Institute of Psychological Research, University of Costa Rica (D. Miranda); Institute of Psychology, University of Göteborg, Sweden (A. Broberg); L. Bernal; P. Brown; S. Jones; C. Larson.

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on long-term consequences of individual differences in physical, social, and affective functioning. In one study, we found stable individual differences in positive and negative responding to a series of emotion-eliciting stimuli as well as in heart rate and vagal tone. Cortisol levels became more individually characteristic over time and varied as a function of diurnal rhythms. Although mothers' perceptions of infant temperament were also highly stable, they were unrelated to observational measures of facial expressiveness or physiological reactivity. Measures of behavioral synchrony in the home predicted differences in the subsequent security of attachment. In a second project, we are studying infant-mother interaction and attachment in infants from upper-middle class Euro-Americans and lower-class Central American families, developing culturally-sensitive criteria for evaluating normative social behavior and development. Regardless of social or ethnic background, mothers spent comparable amounts of time in discrete activities such as feeding, caring for, and playing with their infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01115-06 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Domestic Violence and Evaluation of Children's Testimony

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	K. J. Sternberg	Research Psychologist	LCE, NICHD
	S. Chace	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

Hebrew U, Jerusalem (Greenbaum); Jerusalem Municipality (Garber, Lorey, Cialik); U of Maryland, Baltimore County (Dawud); U of Rochester (Cicchetti); Ministry of Social Welfare, Jerusalem (Hovav, Karniel, Eden, Berger); US Air Force (Gerns, Larison, Mollerstrom); Esplin; Hershkovitz; Manor; Redlich.

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program of research involves two projects. The goal of the first is to explore the effects of domestic violence on 8- to 12-year-old children who were either victims of physical abuse, witnesses of spousal abuse, both victims and witnesses, or neither victims nor witnesses. Overall, children who were physically abused by one or both parents reported more behavior problems, more depression, and more negative perceptions of perpetrating parents than did their counterparts in the respective comparison groups. Mothers reported that their children had the highest levels of problematic behavior when spouse abuse had occurred, whereas children reported the highest levels of problematic behavior when they themselves had been abused. Fathers' and teachers' perceptions of children's problematic behavior were unrelated to the children's experiences of domestic violence.

The goal of the second study is to assess the field reliability and validity of a system for eliciting and assessing statements by children who are alleged to have witnessed sexual abuse. Sites around the United States and in Israel are providing video or audio tapes of child interviews along with case files to establish case facts independent of the statements. Interjudge and test-retest reliability of Criteria-Based Content Analysis (CBCA) techniques were examined and revealed high reliability for total scores and some individual criteria. Some of the other criteria were revised in light of this preliminary research and reliability has been established. The study will also provide basic data on the development of verbal abilities and children's memories of real-life events and generate information about the relationship between interviewer style and quality of information provided by the child.

037

NOTICE OF INTRAMURAL RESEARCH PROJECT | Z01 HD 01116-06 LCE

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Patterns of Childrearing Across Cultures and Ecologies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. E. Lamb	Head, SSED	LCE, NICHD
	C. Bassen	NRSA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
U. Osnabruck, W. Ger. (Keller); U. of NC, Greensboro (MacKinnon); U. of MD (Dawud, Serpell); Ecole Normale, Cameroon (Nsamenang); Hebrew University, Jerusalem (Greenbaum).

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.7

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work on this project involves a number of studies in a variety of cultures. The overall objective is to explore the ways in which developmental environments can be described by variations in physical and social ecologies, especially in terms of parental beliefs, values, and practices, and to assess how differences in these domains affect children's development. In one study, West African parents were interviewed about their perceptions, values, expectations, responsibilities, and practices. Initial results showed that urbanicity, generation, and gender independently influenced the replacement of traditional endogenous beliefs by those of western origin. A related study now in preparation is designed to explore generational differences in parents' perceptions of gender differences and their significance among Moslem Palestinians. In a third study, researchers are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way that parents and children interact, with particular focus on the development of aggressive behavior. Mothers and sons interacted more aggressively when they had negative attributions of one another. Boys who behaved negatively with their mothers were also more aggressive with peers. In the fourth study, interviews will be developed to assess gender differences in self-perceptions of young adolescents. Studies will then be designed to explore the antecedents and correlates of different styles of self-perception.

042

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01117-06 LCE

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Hospitalization Experience: Children's Coping with the Stress of Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. H. Bornstein	Head	LCE, NICHD
J. L. Genevro	NRC Biotech Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research examines age-related differences in children's understanding of and reactions to a brief stay in the hospital for elective surgery. The research integrates the adult stress and coping literature with that on changes in children's cognitive capabilities as they mature, as well as with the behavioral pediatrics literature pertaining to preparing children for hospitalization.

04

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01118-06

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Latent Behavioral Effects of Diverse Forms of Caregiving in the First Year of Life

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head	LCE, NICHD
N. F. Gist	Research Psychologist	LCE, NICHD
O. M. Haynes	Statistician	LCE, NICHD
S. Linnemeyer	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section of Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

052

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01119-06 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mother-Infant Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
J. Suwalsky	Research Psychologist	LCE, NICHD
K. Painter	Research Psychologist	LCE, NICHD
F. Fivel	Research Psychologist	LCE, NICHD
C. Andreassen	NRC Fellow	LCE, NICHD
A. O'Reilly	IRTA Fellow	LCE, NICHD
C. Rahn	Research Psychologist	LCE, NICHD
M. Haynes	Statistician	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.4	2.7	2.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates endogenous and exogenous factors that contribute to the development of cognitive and social competencies in children during the first four years of life. Before children are old enough to enter formal social learning situations, nearly all of their experiences stem directly from interactions they have with their primary caregivers. The present study focuses on the extent to which several maternal characteristics (age, employment status, parenthood status, SES, parity, as well as type of substitute care experienced during mother's employment) influence observed relations between caregiving on the one hand and child cognitive and social competencies on the other. In this longitudinal study, subjects are visited when they are 5, 20, and 48 months old. At 5 months, mother-infant interaction is observed. At 20 months, measures of toddler functioning (i.e., play competence, language development, and social adaptation) and maternal behavior (i.e., play and I.Q.) are obtained. At 48 months, the follow-up examines diverse children's abilities (representational competence, problem solving, reasoning skills, mathematical ability, language use and discourse, and selected aspects of generally adaptive behavior).

053

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01120-06 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Parenting and Infant Activity: A Multiculture Perspective

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
O. M. Haynes	Statistician	LCE, NICHD
C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

ARGENTINA (deGalperin and Berti); BELGIUM (Vyt); FRANCE (Pêcheux); ISRAEL (Maital); ITALY (Venuti and Lis); JAPAN (Azuma, Ogino, and Toda)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiences in infancy are acknowledged to affect the course and outcome of social and mental development in childhood, and they are credited for some of the distinctiveness of culture. More specifically, cross-cultural developmental studies have historically shown that variations in childrearing styles typically have implications for children's later cognitive performance and interpersonal behavior. Home environments are thought to reflect larger cultural values, beliefs, and customs, and many social theorists have contended that the family generally, and the mother-infant relationship specifically, may be vital to development of the individual and basic to the organization of the culture. As a result, investigators have frequently studied infancy and mother-infant interaction in attempts to address questions about the origins and development of cultural identity. Of course, each society has evolved patterns of childrearing adjusted to its own special demands. Some examples of cross-cultural comparison include: It is widely held that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Previous study on the nature of infant development in Israeli kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infant and mother -- vary markedly from the typical American experience. Contemporary France, Italy, and America are relatively similar in terms of industrial level, educational attainment, and living standards, yet these societies differ considerably in terms of history, sociology, and culture. Within-culture comparisons are equally important: Studies of development in Belgium, offer answers to the question of how being reared in a monolingual versus bilingual environment affects cognition, communication, and socioemotional development. Argentina contrasts middle-class with extremely poor rearing conditions, all in a South American setting virtually unresearched. What differences exist in parenting and in infant activity within and across these cultures? One purpose of this project is to identify similarities and differences in the childrearing ecologies of Argentine, Belgian, French, Israeli, Italian, Japanese, and U.S. American infants.

063

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01122-06 LCE

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Longitudinal Assessment of Children's Mental and Social Abilities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein — Head, CFRS — LCE, NICHD

COOPERATING UNITS *(if any)*
Infancy Studies Program, New York University (Tamis-LeMonda)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research continues a longitudinal study of mental and social development in a cohort of children observed repeatedly between 2 months and 4 years of age. The premise of this research is that learning in childhood occurs primarily within an interpersonal context; thus, a central goal is to describe how diverse infant and maternal behaviors relate to the ontogeny of central dimensions of children's mental and social competencies. Children participating in the assessment constitute a longitudinal cohort seen at 2, 5, 13, 20, and 48 months of age. Major objectives of this research are to study stability, continuity, and interaction in mental and social development over the first years of life. One notable aspect of the follow-up is a test of preschooler cognitive and emotional development that goes beyond IQ to assess a wide range of children's diverse interests and capabilities: These procedures assess natural science ability, bodily-kinesthetic skills, musical talents, distinctive styles of work, as well as linguistic and logical-mathematical capabilities. Because traditional psychometric measures of intelligence at preschool age typically sample from a narrow range of mental abilities, such measures are limited in terms of the information they provide about the possible relevance of antecedent variables, and they are also restricted in terms of the outcome variables they successfully predict. In contrast, the new assessment battery samples from a wide range of preschoolers' capabilities and interests and thus affords richer opportunities to respond t many theoretical and pragmatic questions that surround central issues of mental and social development.

074

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01123-03 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Correlates and Neural Mechanisms of the Infant Cry and Related Vocalizations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. D. Newman	Head, DNS	LCE, NICHD
K. Moody	IRTA Fellow	LCE, NICHD
J. Norcross	Pre-IRTA Fellow	LCE, NICHD
C. Garrison	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Department of Zoology, University of Maryland (C.S. Carter); Yale University School of Medicine (L. Mayes); Laboratory of Clinical Science, NIMH (T. Insel)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Developmental Neuroethology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies use neuroethological and ethopharmacological methods to investigate the mechanisms involved in the production and perception of the isolation call and related vocalizations in nonhuman primates. They provide a model for understanding the neurology and physiology of vocal expressions of affect in human infants and the impact of crying on listeners. Major findings this year were as follows: 1. Receptors for oxytocin were revealed with autoradiography in portions of limbic, striatal and olfacto-striatal regions of the brain of common marmosets. In marmoset neonates, portions of the cerebral cortex were labelled, as well, suggesting that oxytocin receptor distribution in the brain changes during development. 2. Systemic administration of an alpha-adrenoreceptor antagonist, prazosin, to adult common marmosets resulted in an increase in territorial behavior and a decrease in affiliative behavior (grooming and isolation calls). A parallel study using adult squirrel monkeys also found that prazosin decreased isolation calling. These findings suggests that the alpha-adrenoreceptor plays a role in regulating behaviors related to social affiliation and territorial defense. 3. A follow-up study of earlier work of the role of cholinergic mechanisms in mediating defensive behavior in squirrel monkeys confirmed that adult females respond more strongly to mildly alarming stimuli than adult males. A full dose response curve for benactyzine HCl, a cholinergic receptor antagonist, was established for males and for females, and indicated that the dose/response curves for both sexes are similar, but that females have a lower threshold dose for exhibiting drug-related increases in alarm behavior.

080

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01124-03 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Experiential Influences on the Development of Primate Vocal Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. D. Newman	Head, SDN	LCE, NICHD
K. Moody	IRTA Fellow	LCE, NICHD
J. Norcross	Pre-IRTA Fellow	LCE, NICHD
C. Garrison	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Department of Linguistics and Cognitive Sciences, Brown University (Fitch)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Developmental Neuroethology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the roles of genetic mechanisms and individual experience in the development of normal vocal behavior in nonhuman primates and human infants. During the past year new findings were obtained from 4 sets of studies, which are summarized below.

1. Development of vocal reptertoire in marmosets: significant contextual differences in the production rates of two distinctive vocal patterns (scream and tsik calls) were found in infant marmosets.

2. Infant retrieval in common marmosets: an important role for recent experience was found in studies of adult/older sibling retrieval of marmoset neonates. Adults and subadults without neonates present in their family group failed to retrieve 1-4 week-old infants, whereas adults and subadults with infants present in their family groups retrieved infants with latencies no greater than 180 seconds.

3. Acoustic differentiation of recruitment screams in rhesus macaques: a group of 35 rhesus macaques who had no previous contact with feral individuals produced agonistic scream vocalizations with the same acoustic subtypes and in the same contexts as feral rhesus macaques living on Cayo Santiago Island off Puerto Rico. This suggests that production and context-dependent usage of this subclass of vocalizations is under genetic control.

4. Development of affect during social separation in infant rhesus mac[illegible]es: a positive correlation between heart rate and scream rate and a negative correlation between heart rate and coo rate was found during 30 min. social separations at 3, 4, and 5 months of age.

5. Maturation of social behavior in common marmosets: longitudinal observations of juveniles in their natal family groups and following pairing with an opposite=sex peer revealed significant context differences in vocal play, and sexual behavior, and few sex differences in behavior in either context.

08

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01125 LCE

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychophysiological Substrates of Cognitive Processing and Socioemotional Expression from 2 to 36 Months

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
S. A. Linnemeyer	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This longitudinal study approaches child development through the analysis of autonomic function, cognitive processing, and socioemotional expression at 2, 5, 13, 18, and 36 months in healthy term infants. In relation to these areas, specific behaviors of the mother (autonomic, intellectual, and interactional) are examined. A combination of physiological assessment, psychometric measurement, and observational techniques is utilized in the study to examine comprehensively the roles of cognitive processing and socioemotional expression in early child development.

08:1

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00705-11 LCMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional organization of the nerve terminal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.T.Russell Head LCMN, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER.
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been cancelled.

00i 01i

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00707-09 LCMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological Studies of Synaptic Transmission In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.L. Mayer	Visiting Scientist	LCMN, NICHD
Others:	M. Benveniste	Senior Staff Fellow	LCMN, NICHD
	K. Partin	NRC fellow	LCMN, NICHD
	D. Patneau	Senior Staff Fellow	LCMN, NICHD
	L. Wong	IRTA Fellow	LCMN, NICHD
	C. Winters	Chemist	LCMN, NICHD

COOPERATING UNITS *(if any)*

Dr. A. Buonanno, LDN, NICHD, NIH. Professor J.C. Watkins, Department of Pharmacology, University of Bristol, UK.

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section of Neurophysiology and Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This section investigates excitatory amino acid receptor mechanisms in the vertebrate CNS, utilizing electrophysiological and molecular biological techniques. Fast perfusion systems are used for concentration jump application of agonists and antagonists to cells and membrane patches under voltage clamp; preparations in use include primary cultures of hippocampal neurons and glial progenitor cells, the CG-4 glial cell line, acutely dissociated sensory neurons, transfected cells and oocytes expressing recombinant receptors. Trapping of agonists at NMDA receptors during ion channel block by amino acridines was demonstrated using voltage jumps in agonist free solutions allowing blocked channels to open and conduct in the absence of agonist. Selectivity for allosteric modulation of the kainate and AMPA-preferring classes of non-NMDA receptors by concanavalin A and cyclothiazide was revealed using recombinant receptors, in conjunction with analysis of the response of native receptors in hippocampal and DRG neurons. In glial progenitors, analysis of the response to these modulators suggests coexpression of both families of glutamate receptors. Structure activity analysis for willardiine action at DRG and hippocampal neurons suggests a hydrophobic pocket in the agonist binding site for kainate but not AMPA receptors.

002

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01203-03 LCMN

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Intracellular signalling in astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dr. J. T. Russell	Head, Section on neuronal secretory systems	LCMN, NICHD
Others:	Ms. L. A. Holtzclaw	Biologist	LCMN, NICHD
	Dr. C. Shepperd	NRC Fellow	LCMN, NICHD
	Dr. P. Holbrook	Senior Staff Fellow	LCMN, NICHD

COOPERATING UNITS (if any)

Dr. S. Yagodin	Visiting Scientist	LNP, NINDS
Dr. V. Gallo	Visiting Associate	LCMN, NICHD
Dr. A. Fatatis	Guest Worker	LCMN, NICHD

LAB/BRANCH
Laboratory of Cellular and Molecular Neurobiology

SECTION
Section on Neuronal secretory systems

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this program is to understand the principles, and mechanisms that govern communication between astrocytes and between astrocytes and neurons. Although neurons are the central players in cellular communication and information processing in the brain, recent discoveries of glial cell types, excitability and signalling properties have shown that glial cells may play an important role in long distance signalling in the brain. During embryonic development, release of specific humoral signals from glial cells are important for neuronal survival and development. In the adult, synaptic activity results in intracellular calcium signals in astrocytes closest to the synaptic fields. The cellular processes involved in this calcium based excitabilility resulting in long distance signalling in glia are not understood. Nor are the mechanisms that underlie calcium w ve propagation in astrocytes. Methods have become available for obtaining relatively pure cultures of different types of astrocytes, as well as mixed neuronal and glial cultures. Methods are also available for light microscopic analysis of cells in slice preparations. Fluorescence microscopy with digital image processing will be used as the technique to monitor intracellular signalling in astrocytes in order to describe specific modes of cellular signalling. The intracellular calcium changes in response to activation of various neurotransmitter receptor systems on astrocytes isolated from different regions of brain will be investigated. The spatio-temporal characteristics of the receptor mediated intracellular calcium signals will be analysed in detail with the view to understanding the underlying mechanisms and processes.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01204-02 LCMN

PERIOD COVERED

October 1, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Receptors for FMRFamide-related Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dr. Gilbert J. Chin Senior Staff Fellow LCMN, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been cancelled.

022

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 02000-02 LCMN

PERIOD COVERED

October 1, September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurotransmitter Receptors in Glia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. :	V. Gallo	Visiting Scientist	LCMN, NICHD
Others:			
	M.M. Molne	Visiting Fellow	LCMN, NICHD
	M. Pende	Adjunct Scientist	LCMN, NICHD
	P. Wright	Senior Lab Technician	EBON Contractor

COOPERATING UNITS *(if any)*

Child Study Center, Yale University, New Haven, CT (Flora Vaccarino)

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Unit on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
20/12	9/12	11/12

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The expression and molecular properties of neurotransmitter ligand-gated ion channels in glial cells were studied in order to understand their function in the brain. Studies were carried out to A. characterize the molecular structure and functional properties of glutamate-gated ion channels during development and B. to determine the effects of activation of these receptor channels in glia.

A. Molecular and functional properties. Primary cultures of rat oligodendrocyte progenitors (O-2A) and the primary cell line CG-4 derived from rat O-2A cells were used as model systems. CG-4 and primary O-2A cells develop into oligodendrocytes *in vitro* similarly to O-2A progenitors *in vivo*. CG-4 and primary O-2A progenitors express the stem cell intermediate filament protein nestin at early developmental stages, followed by the sequential expression of the antigens recognized by the oligodendrocyte-specific antibodies O4 and O1. Patch-clamp recordings revealed that non-NMDA receptor agonists, including kainate, evoked rapidly desensitizing responses in oligodendroglial cells at all developmental stages. Northern blot analysis of total mRNA from O-2A progenitors and oligodendrocytes showed expression of kainate- and AMPA-preferring subunits. Pharmacological experiments revealed two distinct receptor populations with a 50-fold difference in affinity for kainate and selective block of desensitization at AMPA-, but not kainate-preferring receptors by the modulator cyclothiazide. B. Effects of activation of glutamate channels. Fura-2 fluorescence measurements showed that kainate and glutamate caused a fast rise in intracellular Ca^{2+} that was blocked by the antagonist DNQX. The rise of Ca^{2+} was determined to be due to transmembrane influx, mostly through voltage-dependent Ca^{2+} channel. The activation of glutamate receptors in cells of the oligodendroglial lineage also caused a selective increase in the mRNA of the immediate early gene NGFI-A.

The significance of the project lies in the molecular and functional characterization of glutamate-gated channels in glia and in the understanding of their physiological role.

023

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HD1205-01LCMN

PERIOD COVERED

April 1 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Characterization of Hippocampal Inhibitory Interneurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI: C.J. McBain Head LCMN NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section on Cellular and Synaptic Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reasearch project is directed towards studying the physiological and pathological properties ot neurotransmitter-gated ion channels and voltage-gated channels on identified inhibitory interneurons of the hippocampus. The high potassium model of hypersynchronous, epileptiform activity was used to study the cellular elements responsible for seizure initiation and maintenance in the hippocampus. Patch clamp electrophysiology was used to record from inhibitory interneurons located within the hippocampus slice preparation. Elevation of the potassium ion concentration bathing the hippocampal slice resulted in a direct excitation of all inhibitory neurons. These cells were entrained by the ongoing synaptic activity and participated in the burst-firing discharg of the neuronal population. Antagonists of the metabotropic glutamate receptor reversibly abolished the burst firing observed in these neurons, an effect mimicked by antagonists of haem oxygenase. Blockade of these receptors was without effect on the ongoing inhibitory and excitatory synaptic currents received by the interneuron population. This data suggests that the involvement of inhibitory interneurons in seizure episodes are dependent on the activation of the metabotropic glutamate receptor population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01301-11 LDMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Immune Response to Polysaccharide-Protein Conjugate Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Rachel Schneerson Research Medical Officer LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)*

SUNY Downstate Medical Center, N.Y. (G. Schiffman); Charlotte Memorial Hospital, N.C. (J.C. Parke, Jr.); University of Goteborg, Sweden (T. Lagergard); LCI, NIAID (J. Bennett); LMB, NCI (I. Pastan, D. Fitzgerald); BMS, NICHD (D. Bryla); LID, NIDR (J. Keith); LCMB, NIDDK (J. Shiloach); National University Hospital, Iceland (I. Jonsdottir).

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.5	4.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The age-related and T-independent properties of polysaccharides, including capsular polysaccharides and detoxified lipopolysaccharides, limit their usefulness (effectiveness) as vaccines for infants, young children and patients with immunologic deficiencies. Synthetic schemes for covalently binding the polysaccharides of pathogenic bacteria to medically useful proteins have been designed to improve the immunologic properties of these protective antigens. Polysaccharide-protein conjugates have been synthesized for pneumococcus type 6B, pneumococcus type 14, the poly α-(2->8)-D-NeupNAc the capsular polysaccharide of both Group B meningococcus and Escherichia coli K1, Shigella sp., Haemophilus influenzae type a, Cryptococcus neoformans and Staphylococcus aureus. Evaluation of these conjugate vaccines is at various stages, from laboratory standardization to Phase 3 trials of efficacy. In addition, polyribitol phosphate, a polysaccharide cross-reactive with H. influenzae type b has been isolated and its characterization and use as an addition to the current licensed conjugate vaccines for this pathogen is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01304-11 LDMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protective Effect of LPS Antibodies Against Shigella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.B. Robbins Chief LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)*

Pasteur Merieux Serums & Vaccins, France (D. Schulz); Walter Reed Army Institute of Research, Washington, D.C. (J. Sadoff, D. Taylor); Israeli Defense Force, Israel (D. Cohen, S. Ashkenazi); Sheba Medical Center, Israel (J. Passwell); LMC, NIDDK (C.P.J. Glaudemans, P. Kovac); BMS, NICHD (D.A. Bryla); LCDB, NIDDK (J. Shiloach).

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enteric diseases caused by Shigella spp. (shigellosis) remain a serious and frequent cause of morbidity and mortality throughout the developing world especially in infants and children. Treatment of shigellosis has become difficult because most strains of Shigella are resistant to commonly used antibiotics. Shigellosis is the major cause of dysentery in infants and children with its serious complications: death or decreased growth and weight loss. In addition, shigellosis occurs in healthy individuals under conditions of crowding, such as in armed forces recruits, or in institutions caring for debilitated patients. Despite a century of study after its discovery, there are no licensed vaccines for prevention of shigellosis. This deficiency is due to our ignorance of what is/are the host protective immune moiety(ies) and a lack of a valid animal model for shigellosis. We have hypothesized that serum IgG antibodies to the O-specific polysaccharide of Shigella will confer protective immunity to shigellosis. The LPS of Shigella has been isolated and detoxified by acid hydrolysis. The detoxified LPS has been bound to several carrier proteins and Phase I and Phase 2 clinical studies have shown these investigational vaccines to be safe and immunogenic in adults. The level of conjugate vaccine antibodies is equal or higher than those in patients following shigellosis. In addition, the repeating unit of the O-specific polysaccharide of Shigella dysenteriae type I (Shiga) has been synthesized and the immunodominant epitope has been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01308-11 LDMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjugate-Induced Polysaccharide Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.C. Szu Research Chemist LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)*

University of Missouri-Columbia, Missouri (R. Finkelstein); WRAIR, District of Columbia (D. Taylor, A. Trofa); OD, NIDDK (E. Kabat); LMC, NIDDK (P. Kovac); Marine Biological Laboratories, Massachusetts (N. Wainwright).

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.4	4.4	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence is accruing that serum antibodies to surface polysaccharides, including lipopolysaccharides of Gram-negative organisms, confer protective immunity. The Vi has been licensed by the World Health Organization and the FDA as a vaccine for typhoid fever. To overcome the age-related and T-independent properties of this capsular polysaccharide, the Vi was bound to medically-useful proteins by several synthetic schemes. A Phase I study confirmed the improved immunologic properties of Vi as a conjugate made with the B-subunit of the heat-labile toxin of Escherichia coli or a recombinant Pseudomonas aeruginosa exoprotein A. The Vi was bound to the protein by a bifunctional hetero linker, SPDP. Attempts were made to bind the Vi to a protein with adipic acid dihydrazide. A semisynthetic Vi was prepared by derivatizing pectin, a plant polysaccharide whose repeat unit is α-D-(1->4)GalA, with acetic anhydride. The product was identical to the Vi with the exception that the C-2 of the treated pectin is O- rather than N-acetylated. The immunologic properties of the di-O-acetylated pectin have been compared to the Vi. The structure of the Vi, especially the interaction of the carboxyl and the O-acetyl on C-2, was investigated by potentiometric titration, circular dichroism and reaction with a bulky organic reagent. The data fitted the conclusions drawn from construction of a space filling model in which the surface of the Vi was occupied by the acetyls which covered the carboxyls. Clinical studies confirmed the immunogenicity of a conjugate composed of the detoxified LPS of Vibrio cholerae. The serum antibodies are being analyzed the immunoglobulin composition of their vibriocidal activity. A new sero type cholera 0139 causing epidemics in India is under investigation for its LPS structure and possible cross-reactivity with other cholera. Another LPS, that of E. coli 0157, has been purified and bound to the exotoxin A of Clostridium welchii C (pig bel toxin). The resultant conjugate was immunogenic in mice and a lot suitable for clinical study is under synthesis with the objective of evaluating its safety, immunogenicity and ultimately, effective in preventing enteritis caused by this pathogens with especial reference to the complication of the hemolytic uremic syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00047-24 LDN

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical Studies of Neurons and Other Cell Types

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Brenneman	Head	LDN,NICHD
Others:	R. Avidor	Visiting Fellow	LDN,NICHD
	J. Hill	Expert	LDN,NICHD
	S. McCune	IPA	LDN,NICHD
	I. Gozes	Guest Researcher	LDN,NICHD
	D. Warren	Bio. Lab Tech.	LDN,NICHD

COOPERATING UNITS (if any)
Biol. Psych. Br., NIMH (E. Sternberg); Dept. Organic Chem., Weizmann Inst. (M. Fridkin); Dept. Neurol., Univ. Kansas (B. Festoff); Lab Exper. Neuropath. NINDS (P. Gressens); Dept. Biochem. George Washington Univ. (T. Moody). Dept. of Immunol. George Washington Univ. (T. Phillips).

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Developmental and Molecular Pharmacology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Growth Factors and cytokines released by vasoactive intestinal peptide (VIP) were studied as mediators of neuron-glia interactions that regulate the survival of developing neurons and growth of mammalia embryos. Primary cell cultures derived from murine central nervous system were utilized to further characterize the molecular mechanism of VIP neurotrophism. The newly discovered growth factor, Activity Dependent Neurotrophic Factor (ADNF) was shown to potently increase the survival of neurons from cerebral cortex and hippocampus and to prevent the cell death associated with the envelope protein from the human immunodeficiency virus. Interleukin-1-alpha was shown to be released from astroglial cells by low concentrations of VIP. Treatment with higher concentrations of VIP also elevated IL-1 alpha mRNA. Studies on the developmental role of VIP were extended to growth regulation of whole embryo cultures. After four hours incubation, VIP increased somite number, embryonic volume, DNA and protein content, and the number of cells in S-phase. The VIP antagonist developed in our laboratory inhibited the VIP-mediated increments in growth in the CNS, but only attenuated the increases in growth in non-neural tissue. In vitro autoradiography revealed GTP-sensitive and GTP-insensitive VIP receptors which were differentially regulated in VIP-antagonist-treated embryos. The growth promoting effects elicited by VIP were not observed with Pituitary Adenylate Cyclase Activating Peptide. The distribution of both VIP and its receptors were investigated in the developing embryo. VIP mRNA was not observed in embryonic mice or rats whereas VIP receptors were abundant and dynamic with development. The role of maternal VIP in mediating embryonic growth was investigated. Radiolabeled VIP injected into uterine vessels was recovered in fetal brain.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00056-18 LDN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Y. Peng Loh	Section Chief	LDN, NICHD
Others:	LePing Pu	Visiting Fellow	LDN, NICHD
	Theodore Friedman	Medical Staff Fellow	LDN, NICHD
	Ana-Maria Bamberger	Visiting Fellow	LDN, NICHD
	David Cool	NRC Associate	LDN, NICHD
	Niamh Cawley	PRE-IRTA	LDN, NICHD
	Winnie Tam	Microbiologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Lab. Microbiol. Ecology NIDR (V. Gordon); Endocrinology and Reproduction Research Branch (H-C Chen); Univ of Auckland, New Zealand (N. Birch); Brown Univ (E. Nillni, I. Jackson); Emory Univ (B. Noe)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
6.0	4.0	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The signal involved in the targeting of pro-opiomelanocortin (POMC, pro-ACTH/endorphin) to the regulated secretory pathway was identified. Truncated POMC/CAT constructs transfected into AtT-20 cells indicated that the N-terminal 26 amino acids of POMC which form a hairpin loop stabilized by two disulfide bridges are sufficient and necessary for targeting POMC to the regulated secretory pathway. POMC mutants with the entire 24 amino acid hairpin loop or both the disulfide bridges deleted, failed to be targeted to secretory granules. However, a mutant truncated to 11 amino acids with one disulfide bridge to form a mini-hairpin loop was correctly sorted. These findings indicate that the sorting signal is encoded by a specific conformation. Pituitary secretory vesicle enzymes involved in the processing of POMC were studied. The Ca++ activated aspartic protease from bovine intermediate lobe secretory granules, POMC converting enzyme (PCE; EC 3.4.23.17) which cleaves POMC at paired basic residues has been shown to cleave at a monobasic residue of anglerfish pro-somatostatin II (aPSSII) to generate somatostatin 28. A yeast aspartic protease (YAP3) has been purified and shown to have a similar inhibitor profile, size and specificity for the paired basic residues of POMC and the monobasic residue of aPSSII as PCE. An antibody against YAP3 cross-reacted with PCE on Western blot and in a radioimmunoassay. Immunocytochemistry using this antibody showed staining in cells of the intermediate lobe and anterior lobe where PCE has been found. PCE appears to be homologous to YAP3 structurally and in catalytic activity. Two prohormone processing enzyme candidates, PC1 and PC2 belonging to the subtilisin-related family of serine proteases, were shown to process POMC, protective antigen (PA) of anthrax and pro-TRH correctly at paired basic residues *in vitro*. Use of PA substrates mutated at the tetrabasic cleavage site revealed the importance of the secondary and tertiary structure in dictating the specificity of processing. PC1 and PC2 mRNA have been colocalized with TRH immunopositive neurons by *in situ* hybridization and immunocytochemistry, supporting a role of these enzymes in processing pro-TRH *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00064-17 LDN

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neurobiologic Studies of Neurons and Glia in Cell Culture

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.G. Nelson	Chief	LDN,NICHD
Others:	R.D. Fields	Senior Staff Fellow	LDN,NICHD
	P.-X. Lin	Visiting Fellow	LDN,NICHD
	H.Z. Sheng	Visiting Fellow	LDN,NICHD
	Y. Liu	NRC Fellow	LDN,NICHD
	S. Fitzgerald	Biologist	LDN,NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
7.4	5.4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activity-dependent synapse elimination can be demonstrated in a compartmental tissue culture system in which two populations of cholinergic neurons converge on and innervate a common target population of muscle cells. This synapse elimination can be blocked by the inhibitor of serine and thiol proteases, leupeptin, at concentrations of 10 nM and greater. The more specific blocker of serine proteases, aprotinin, fails to block elimination as does the thiol protease blocker cystatin. Hirudin is a highly specific inhibitor of the serine protease, thrombin and hirudin does block synapse elimination at 1 nM concentration (aprotinin does not block thrombin). Protease nexin I (PNI), a naturally occurring protease inhibitor (identical with glial derived nexin or GDN) also blocks thrombin and prevents activity-dependent synapse elimination. These data strongly implicate thrombin in the process of synapse elimination. Kinetic analysis of internal calcium, $[Ca^{++}]_i$, shows that single action potentials produce a larger increase in $[Ca^{++}]_i$ than do the individual action potentials of rapid (10 Hz) pulse trains. The peak $[Ca^{++}]_i$ reached during repetitive bursts of action potentials, however, is much higher than with single action potentials. These kinetic data are useful in interpreting the mechanisms coupling electrical activity to gene expression. Retinal ganglion cells (RGC) in living chick embryos can be labelled with horseradish peroxidase. The growth cones of the axons of these RGC are visualized with this technique and exhibit dramatic changes at different places in the optic pathway (nerve, chiasm and tract). This preparation should allow evaluation of the effects of different manipulations of the visual system (electrical stimulation, protease inhibitors, cell adhesion molecule antibodies) upon growth cone morphology and axonal projection patterns.

016

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00094-23 LDN

PERIOD COVERED
October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pineal Regulation: Environmental and Physiological Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.C. Klein	Head	LDN, NICHD
Others:	Nicolas Schaad	Visiting Fellow	LDN, NICHD
	Steven Coon	NRC Fellow	LDN, NICHD

COOPERATING UNITS *(if any)*
Georgetown University (M.A.A. Namboodiri); University of Geneva (N. Schaad); University of Giessen (H. Korf)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS.	PROFESSIONAL	OTHER
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The regulation of the pineal gland by environmental and physiological factors is analyzed, exclusive of transmembrane and intracellular regulatory mechanisms (see Z01 HD 00095-22 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. An area of special current interest is proteins involved in transmembrane signal processing. These include G-proteins, MEKA and the S-antigen. These studies indicate that MEKA and the S-antigen are absent before birth and appear rapidly during the first postnatal week in the rat. In contrast, Gβ is present at adult levels prior to birth. In the adult, it has been found that the amount of MEKA and the phosphorylation state of this enzyme are regulated by environmental lighting. Total MEKA increases at night; phosphorylation also increases at night in the dark and the protein is rapidly dephosphorylated by exposure to light. An interesting new finding is that the amount of pineal Gsα is under impressive regulation, increasing following procedures which block neural stimulation. In addition, the large and small splice variants of Gsα exhibit different developmental patterns. It has also been discovered that the developmental appearance of the adrenergic stimulation of pineal cyclic GMP occurs several weeks after that of the adrenergic stimulation of cyclic AMP.

020

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00095-23 LDN

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.C. Klein	Head	LDN, NICHD
Others:	J.L. Weller	Chemist	LDN, NICHD
	P. Roseboom	Biotechnology Fellow	LDN, NICHD
	A. Lin	Visiting Fellow	LDN, NICHD
	N. Schaad	Visiting Fellow	LDN, NICHD
	J. Vanecek	Visiting Scientist	LDN, NICHD
	Z. Naor	Special Volunteer	LDN, NICHD

COOPERATING UNITS *(if any)*

Georgetown Univ (M.A.A. Namboodiri); NEI (T. Shinohara); Univ of Giessen (H. Korf); NCI (W. McBride); NIMH (E. Ginns); LDN, NICHD (J. Russell, J. Hill); Dupont (B. Marrs); Harvard Univ. (T. Babila); NIMH (W. Klee)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
8.0	6.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms involved in the transduction of neural signals and the control of tissue specific gene expression are studied. The pineal and pituitary glands are used as model systems. The details of the chemical and ionic components of transmembrane signalling processing and of neural and tissue specific regulation of gene expression are analyzed. Signal transduction in the pineal is of special interest because cAMP and cGMP are regulated by dual receptor mechanisms which appear to interact at the level of regulation of adenylyl and guanylyl cyclases. One leg of these pathways activates these enzymes via GTP binding regulatory proteins, similar to Gsα. In the area of the neural and developmental control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindol-O-methyltransferase, and in isolating cDNA clones coding for these enzymes. It has been found that three HIOMT mRNA molecules exist, which appear to be the result of alternative splicing; one contains a truncated LINE sequence in the coding region.

The major hormonal product of the pineal gland is melatonin. Melatonin has been found to block GnRH induced increase in $[Ca^{++}]i$ and to block GnRH-induced depolarization. Melatonin appears to act on a subpopulation of GnRH-sensitive cells.

024

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00704-09 LDN

PERIOD COVERED: October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Tetanus Toxin Effects and Localization in Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Neale	Head	LDN,NICHD
	L.C. Williamson	Sr. Staff Fellow	LDN,NICHD
Others:	Linda M. Bowers	Biologist	LDN,NICHD
	S.C. Fitzgerald	Biologist	LDN,NICHD

COOPERATING UNITS *(if any)*: Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration (J. H. Halpern and W.H. Habig); NIDR (S.H. Leppla)

LAB/BRANCH: Laboratory of Developmental Neurobiology

SECTION: Unit on Cell Biology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tetanus toxin is internalized by way of acidic endosomes. Monensin and bafilomycin, agents which disrupt the endosomal pH gradient, protect neurons against the effects of the toxin.

In synaptic terminals poisoned with tetanus toxin, there are twice as many synaptic vesicles aligned at the active zone as there are in control preparations. This finding strengthens the notion that the toxin acts by preventing vesicle fusion with the presynaptic membrane.

Tetanus toxin acts with a different time course on inhibitory and excitatory synapses. Following exposure to tetanus toxin, inhibitory synapses are blocked more quickly and recover more slowly than excitatory synapses.

Neurotransmitter release from neuronal cell cultures has proven to be a reliable assay for the efficacy of anthrax toxin-tetanus toxin fusion proteins.

033

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00708-09 LDN

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphologic Studies of Neuronal and Non-neuronal cells in CNS Cell Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Neale	Head	LDN,NICHD
Others:	A.G. Parfitt	Adjunct Scientist	LDN,NICHD
	L.M. Bowers	Biologist	LDN,NICHD

COOPERATING UNITS *(if any)*
Laboratory of Neurophysiology, NINDS, NIH (T.J. Smith, Jr.); Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration (W.H. Habig and J.L. Halpern).

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Cell Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Axonal, dendritic, or synaptic structures were identified by immunohistochemistry in developing neuronal cultures. In cultures where electrical activity was suppressed, axonal and synaptic structures were more abundant than in active cultures.

A methodology has been developed for the preparation of a multicompartment ventral horn neuron / hindlimb muscle cultures. Functional neuromuscular connections are formed, and the effect of electrical activity on the development and maintenance of such connections can be evaluated physiologically and morphologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00709-07 LDN

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Prevention of Neuronal Deficits Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D. Brenneman Head LDN,NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Developmental Neurobiology

SECTION: Section on Developmental and Molecular Pharmacology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive.

041

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00710-05 LDN

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Glutamate Receptor Expression in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Buonanno	Head	LDN,NICHD
Others:	M. Sasner	IRTA Fellow	LDN,NICHD
	R. Beers	Biologist	LDN,NICHD
	K. Partin	NRC Fellow	LCMN,NICHD
	L. Wong	IRTA	LCMN,NICHD
	M. Mayer	Chief	LCMN,NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Molecular Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glutamate receptors (GluRs), broadly classified in the NMDA and non-NMDA subtypes, have been implicated in simple forms of learning and memory and are the site of action of several psychoactive drugs. Molecular cloning of GluR cDNAs has revealed a large family of distantly related receptor subunits that can be classified into groups by their sequence similarity and/or agonist binding preferences. These groups include: NMDA-R1 and NMDA-R2 A-D (agonist is NMDA), GluR 1-4 (AMPA-preferring), and GluR 5-7 and KA1,2 (kainate-preferring). The genes coding for GluR subunits are differentially expressed in regions of the CNS, and during development. We have begun to isolate and characterize the transcription regulatory sequences of NMDA-R genes, in order to identify the elements that impart developmental and regional-specific expression, and confer activity-dependent regulation. Alternative splicing and RNA editing add further complexity to the different receptor isoforms that can be generated from a single GluR gene, resulting in the assembly of receptors that exhibit marked differences in ion-selectivity and desensitization properties. In the case of the GluR 4 gene, we have identified 6.2 and 4.2 transcripts that code for functional GluR receptors. In addition, we have identified a related abundant 3.0 kb cerebellar transcript that codes for a putative truncated protein missing the 4 transmembrane spanning regions present in other GluR subunits. This protein has been called GluR-4s ("s" for short). Southern and PCR analysis demonstrate that the GluR 4 gene is present in a single copy, and that GluR 4s results from differential RNA processing. The expression of this poly-adenylated transcript is developmentally regulated and predominantly confined to neurons, in contrast to the other GluR mRNAs which are expressed in Bergman glia and astrocytes. In collaboration with Dr. Mayer's laboratory, we have analyzed the differential expression of non-NMDA GluR subunits in dorsal root ganglia (DRG), forebrain and hippocampus. DRG were found to predominantly express kainate-preferring GluR subunits, whereas both families of receptor mRNAs were found in cerebellum and forebrain. Expression of recombinant receptors in *Xenopus* oocytes showed that kainate responses at AMPA-and kainate-preferring receptors are differentially potentiated by Con A and cyclothiazide. Oocytes injected with combinations of kainate-preferring receptors showed similar pharmacological responses to those observed in DRG, whereas those injected with AMPA-preferring subunits elicited similar responses to those found in hippocampal neurons. These results suggest that different families of GluR subunits selectively assemble to form functional receptors.

042

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HD 00711-04 LDN

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Transcriptional Regulation of Skeletal Muscle-Specific Genes by Electrical Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Buonanno	Head	LDN, NICHD
Others:	S. Basu	IRTA	LDN, NICHD
	G. Gibney	NRC	LDN, NICHD
	J. Stauffer	IRTA/MDA Fellow	LDN, NICHD
	L. Anglister	Visiting Scientist	LDN, NICHD
	R. Beers	Biologist	LDN, NICHD
	W. Hayes	IRTA	LDN, NICHD

COOPERATING UNITS (if any)

NEI (Dr. Eric Wourusek)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Molecular Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Motoneuron innervation dramatically influences the phenotypic properties of skeletal muscle. The pattern and frequency of nerve-elicited electrical activity modifies transcription of nicotinic acetylcholine receptor (nAChR) genes, as well as genes coding for myofibril proteins which determine the contractile properties of the muscle fiber. We have identified muscle genes that are either repressed or selectively stimulated by activity; our goal is to elucidate the molecular mechanisms underlying activity-dependent transcriptional regulation. To this end, we previously demonstrated that myogenin, a muscle-specific factor that regulates transcription of nAChR genes in cultured cells, is repressed by innervation and de-repressed by denervation. Changes in myogenin levels in muscle preceded receptor regulation during development and after denervation, suggesting that regulation of myogenin may lie upstream of receptor. Analysis of a 3.7 kb myogenin upstream region in transgenic mice have shown that these sequences confer muscle-, developmental-, and denervation-specific regulation. We have used myoblast implantation experiments to separate sequences that confer tissue and developmental sp · ificity from those imparting the dene-vation response. We analyzed the *cis*-acting elements regulating expression of the troponin I slow (TnI_s) gene, a myofibril protein coding gene that is specifically expressed in slow-type muscle and is up-regulated selectively by "slow patterns" of electrical stimulation. A 6.3 kb TnI_s fragment, containing the first 2 noncoding exons plus upstream sequences, was found to direct transcription specifically to slow-type muscle. Delineation of this region in cultured C2C12 myocytes demonstrated that 200 bp of upstream sequence are necessary and sufficient to confer muscle- and developmental-specific transcription. Based on these sequences, we have begun to clone, and analyze the expression of, putative *trans*-acting factors that may regulate TnI_s transcription. A cDNA coding for a novel member of the *ets*-family, with highest homology to PEA-3, has been isolated and sequenced. The functional properties of this protein are currently under investigation. A rat MEF-2 cDNA was also cloned and sequenced, and its expression during development and in different fiber types is currently being studied. Analysis of the interactions of these families of *trans*-acting factors should help elucidate the molecular mechanisms underlying fiber-type specific regulation of muscle genes by innervation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00712-02 LDN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of phenotypic differentiation and diversity in the developing mammalian CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Agoston	Head	LDN, NICHD
Others:	A. Dobi	Visiting Fellow	LDN, NICHD
	E. Santha	Visiting Associate	LDN, NICHD
	R. Carbone	Adjunct Scientist	LDN, NICHD
	C. Palkovits	Lab Tech.	LDN, NICHD

COOPERATING UNITS *(if any)*
Cooperating Units: Neuropsychiatric Inst., UCLA (J. Waschek); LCB,NIMH (M. Palkovits); Dept. Neurobiology, Duke Univ. (M. Riggott & W.D. Matthew), LMP, NINDS (G. Jakab); LNC, NINDS (A. Donevan & M. O'Donovan); LN, NINDS (B. Andrews), LNC, NINDS, (H. Chin)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Molecular Control of Neurodifferentiation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular regulation of phenotypic establishment was studied during neuronal differentiation through the identification of embryonic-specific trans-factors and their corresponding cis-elements, the role of gene structure, chromatinization and methylation. The rat and mouse enkephalin and VIP genes were isolated, characterized, and used as models of phenotypic genes. Nuclear extracts of different brain regions and peripheral tissues from animals of various ages, developing primary neuronal cell cultures, tumor cell lines and transgene animals were employed as model systems as well. Experiments with cycloheximide showed that phenotypic establishment (embryonic expression of the phenotype) is critically dependent on de novo protein biosynthesis. Mobility shift assays with different fragments of the enkephalin gene cis-elements showed numerous specific complexes exclusively present in certain, but not all, regions of the prenatal brain and absent from adult brain as well as from peripheral tissues (adrenal, liver).We found elaborate, tissue-, cell-and age-dependent binding patterns indicating the existence of large number of embryonic- and cell-specific DNA-binding proteins. Preincubation of nuclear extracts with embryonic-specific monoclonal antibodies recognized several DNA-binding proteins and resulting "supershifts". A Ca2+ -induced "supershift" specific to the fragment of the rat ppENK gene that contair the sequence motif (TG/CA)56 between -614 and -670 positions (presumed Z-DNA) was observed. Gel shift assays with ds(TG)28/(AC)28 (Z-DNA) probe at low Ca2+/Mg2+ concentration revealed the presence of specific binding proteins in nuclear extracts of peripheral tissues (liver, adrenal) which were absent in CNS. Using a variety of techniques it was demonstrated that: a) only the presumed Z-DNA sequence motif (TG/CA)n, but no other random sequences tested, was capable of "DNA-slippage" or sliding, in a Ca2+/Mg2+-dependent manner; b) the sliding was concentration dependent and maximal at the concentration of 1-3 mM; c) this "slippage/sliding" did not require the presence of protein at all; d) the direction of the sliding was strictly unidirectional (3' to 5'). The mouse VIP chromosomal gene was extensively characterized and sequenced and showed the presence of the presumed Z-DNA sequence motif (TG/CA)38 and high homology to the human gene. Immunohistochemical studies on developing DRG neurons showed a temporary, developmental-specific intranuclear localization of CGRP, the neuropeptide involved in noceffector function. The activity-dependent regulation of the synaptic specific phosphoprotein GAP-43 was studied in primary DRG neurons and no activity-dependent regulation was found at the transcriptional level.

056

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01202-6 LDN

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Expression and Function of Neuropeptides During Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Y. Peng Loh	Section Chief	LDN, NICHD
Others:	William Hayes	IRTA	LDN, NICHD
	LePing Pu	Visiting Fellow	LDN, NICHD

COOPERATING UNITS (if any)
Lab Biol Chem, NCI (J Battey), Lab Neurochem, NINDS (S. Wray, J Mill & H. Chin), Dev. Metabolic Neurology Branch, NINDS, (S. Karlsson); Univ of Montreal (J. Chan)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Cellular Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.75	1.75	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of local interactions in the diversification of neuropeptide phenotypes was studied in developing *Xenopus* brain, using *in situ* hybridization histochemistry. A highly localized appearance of pro-opiomelanocortin (POMC) cells was observed in the pituitary and forebrain suggesting the existence of a common POMC precursor that is anterior most in the neural plate. In another neuropeptide phenotype TRH, the pattern spanning adult forebrain, comprising over six different nuclei arise from a dispersed population that is highly regionalized embryonically. Thus a subset of phenotypically distinct neuronal cells develops as a group before distinct forebrain nuclei form, arguing specific neuronal cell fates may be dictated by cell lineage or local induction. The developmental expression of GnRH-I was also studied. Unlike POMC and TRH, GnRH-I was suppressed until metamorphic climax. GnRH cells were first detected after they reached their final position in the preoptic area during the late larval period. A fragment of *Xenopus* PC2, a prohormone converting enzyme was cloned by PCR and the cDNA used in *in situ* hybridization as a second phenotypic marker for neuropeptide cells. PC2 was found to be expressed in a highly localized region specific pattern which included TRH neurons. The expression of a new LIM-class family of homeobox genes was characterized from embryo to adult *Xenopus* CNS. It was found that the Xlim-3 gene was expressed in frog pineal, pituitary, hindbrain and retina. However, it was not expressed in a specific subset of cells in the anterior pituitary or pineal. Xlim-1 was expressed in certain more posterior forebrain nuclei and the expression pattern could include some forebrain cells that express TRH and GnRH, but not POMC. The LIM-class homeobox genes may be involved in the specification of brain cytoarchitecture corresponding to individual brain nuclei including ones expressing specific neuropeptide genes.

06

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01900-03 LME

PERIOD COVERED

October 1st, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Regulation of Differential Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alan Wolffe, Visiting Scientist LME/NICHD

OTHERS

Geneviève Almouzni, Visiting Associate LME/NICHD
Philippe Bouvet, Special Volunteer LME/NICHD
Mary Dasso, Staff Fellow LME/NICHD
Stefan Dimitrov, Visiting Scientist LME/NICHD
Lita Freeman, Special Volunteer LME/NICHD
James Godde, IRTA Fellow LME/NICHD
Jeffrey Hayes, IRTA Fellow LME/NICHD
Nicoletta Landsberger, Special Volunteer LME/NICHD
Timothy O'Neill, Special Volunteer LME/NICHD
Karl Nightingale, Visiting Fellow LME/NICHD
Danielle Patterton, Visiting Associate LME/NICHD
Dmitry Pruss, Visiting Fellow LME/NICHD
Monika Puzianowska-Kuznicki, Visiting Fellow LME/NICHD
Yun-Bo Shi, Senior Staff Fellow LME/NICHD
Donald Stump, IRTA Fellow LME/NICHD
Kiyoe Ura, Special Volunteer LME/NICHD

COOPERATING UNITS *(if any)*

Institut Biologie Animale Lausanne, Switzerland (W. Wahli); Univ. Texas, San Antonio (J. Hansen); Hebrew Univ. of Jerusalem (B. Shaanan); LMGR/NICHD (B. Howard); LMC/NCI (M. Bustin); Kyushu University, Japan (T. Nishimoto)

LAB/BRANCH

Laboratory of Molecular Embryology

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
13	13	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our work is focussed on the molecular mechanisms responsible for establishing and maintaining stable states of gene expression during vertebrate embryogenesis. Progress has been achieved in the following key areas:

1. We have demonstrated the necessity of chromatin assembly to be coupled to DNA replication to effect repression of basal transcription in vivo.

2. We have made the first determination of specific histone-DNA contacts within a nucleosome incorporating a functional eukaryotic gene. The information leads to a new model for the nucleosome incorporating an asymmetric interaction of histone H1.

3. We have demonstrated that the developmentally regulated replacement of histone variants during vertebrate embryogenesis has functional significance.

4. The Y-box proteins have been shown to mediate not only the accumulation of mRNA from promoters containing their recognition element, but also to direct translational repression in vivo. This is the first reconstruction of 'masked mRNA' under controlled conditions.

5. The thyroid hormone receptor has been shown to activate genes encoding metalloproteinases and to repress genes encoding intestinal fatty acid binding protein during amphibian metamorphosis.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00066-23 LMG

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control Mechanisms in Temperate Bacteriophage Δ

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Robert A. Weisberg	Section Head (All listed personnel LMG:NICHD)		
Others:	Banik-Maiti, Sarbani	Vis. Fellow	King, Rodney	NRC
	Clerget, Michel	Vis. Assoc.	Sloan, Sieghild	Microbiologist
	Ditto, Mary	IRTA	Szu, Evan	Guest Worker
	Dorgai, Laszlo	Vis. Assoc.	Yagil, Ezra	Courtesy Contract

COOPERATING UNITS (if any)

Department of Biochemistry, Tel Aviv University, Israel (Ezra Yagil); Laboratory of Molecular Biology, NCI (Ding Jun Jin)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Genetics of Lower Eukaryotes

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.8	4.6	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

We have used a gene encoding a stable, biologically active suppressor tRNA as a reporter of transcription. We used this RNA reporter to show that the bacteriophage HK022 Nun protein blocks gene expression by terminating transcription close to the phage λ nut sites, and does not act by destabilizing transcripts or by preventing translation. This reporter system should be useful for other investigations in which it is necessary to distinguish between transcript termination and transcript stability. We have continued our studies on the mechanism of transcription antitermination in bacteriophage HK022. Measurements of terminator readthrough in vivo and in vitro suggest that antitermination requires a Zinc-binding domain in the ß' subunit of E. coli RNA polymerase, and a specific site that lies upstream of terminator signals in the two early transcription units of the phage. The protein domain has been identified by a cluster of mutations that prevent antitermination, but otherwise leave polymerase activity nearly unaltered. We have found hat these mutations also alter expression of several bacterial transcription units, and we are isolating and characterizing the genes involved. The integrase proteins of phages λ and HK022 are closely related site-specific recombinases that recognize different nucleotide sequences in their substrates, the attachment sites of the two phages. To locate the determinants of specificity within the sites and proteins, we have developed a new excision assay for rapidly testing the activities of mutants. We find that a chimeric protein that contains only 12 amino acid residues of HK022 origin has nearly complete HK022 specificity. These residues lie between positions 279 and 330, overlapping the active site of the protein. Reciprocal chimeras do not have the reciprocal phenotype, but instead recognize both types of sites. We also found that pairs of sites with 4 identical core regions are less active than wild type sites. We propose a model in which cores are recognized by an Integrase oligomer, and that interactions between the protomers indirectly affect DNA site recognition.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00067-25 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Integration of Macromolecular Synthesis in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Cashel, M.D., Ph.D.	Section Chief	LMG:NICHD
Others:	D. Gentry, Ph.D.	NRC Fellow	LMG:NICHD
	J. Hernandez, Ph.D.	IRTA Fellow	LMG:NICHD
	G. Glaser, M.D., Ph.D.	Visitor	LMG:NICHD
	R. Rotz	Bio. Lab. Tech	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. R. R. Burgess, McArdle Lab. for Cancer Research, Madison, WI 53706
Dr. G. Glaser, Hadassah Medical School, Jerusalem, Israel

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project continues to involve attempting to understand how changes in gene expression are coordinated when cell growth is limited by nutrient availability. Escherichia coli has been studied with specific focus on amino acid and energy source availability as signals triggering cellular accumulation of analogs of GDP and GTP that bear 3'-pyrophosphate residues, abbreviated as ppGpp and pppGpp, respectively, or (p)ppGpp collectively. Estimates of regulatory effects of (p)ppGpp on gene expression and cell physiology gained from either the ability to manipulate (p)ppGpp concentrations at will or constructing mutant strains lacking the ability to form (p)ppGpp. This year we have learned that our interests must be extended to include stationary phase and polyphosphate metabolism.

A monoclonal antibody, obtained from the Burgess laboratory, has been used to demonstrate that (p)ppGpp activates accumulation of a stationary phase-specific sigma (sigma-S) factor encoded by the rpoS gene. A strong dependence on (p)ppGpp was shown when growth was limited for either amino acids, glucose, phosphate, or when complex media was exhausted. Conversely, induction of (p)ppGpp, without nutrient limitation, rapidly induced sigma-S levels at least 50-fold. Since many stationary phase-specific genes show expression dependent upon a functional rpoS gene, this implies that (p)ppGpp can also indirectly activate these genes.

This year's project work has also led to discovering that ppGpp-deficiency destabilizes sigma-70 (encoded by rpoD) complexes with core RNA polymerase (subunits rpoA-C). This property is reversed by rpoD mutants selected as suppressors of (p)ppGpp-deficiency. Mutant behavior hints that (p)ppGpp alters global transcription by affecting the competition between core and alternative sigma factors. This notion is strengthened by sequences of the rpoD mutations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00068-22

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Factors Influencing Genetics Transcription-Initiation and Termination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert J. Crouch	Research Chemist	(All listed LMG:NICHD)		
Others:	D. Ennis	Senior Staff Fellow	X. Zahn		Vis. Fellow
	S. Cerritelli	Vis. Associate	Bundara-Freeland		Microbiologist
	W. Ma	Vis. Fellow	L. Gold		Biolab Tech
	Y. Tozawa	Vis. Fellow			

COOPERATING UNITS *(if any)*

J. Levin, LMG, NICHD, NIH; R. Woodgate, Section on Viruses and Cellular Biology, NICHD, H.C. Chen ERRB, NICHD, L. Neckers, Clinical Pharmacology Branch, NCI, S. Stahl, Protein Expression Laboratory, OD OIR T. Uchida, Mitsubishi-kasei Institute

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Formation of RNA

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.25	4.33	0.82

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

RNases H are associated with retroviral reverse transcriptase (RT) and are active during polymerization. Cellular RNases H probably function in various processes, including selection of origins of initiation of DNA replication. One example of a cellular RNase H is the *S. cerevisiae* RNase H1, a 39 kDa protein with the C-terminal domain carrying the RNase H activity and the amino-terminal region is a double-stranded RNA binding domain. The RNase H activity of reverse transcriptase of HIV is a very low specific activity enzyme compared to cellular enzymes of *E. coli* and *S. cerevisiae*. Substitution of one region of the HIV RNase H with the homologous portion of the *E. coli* protein has been found to increase the specific activity of the HIV RNase H without altering its (limited) specificity. Conversely, expressing the high specific activity *E. coli* RNase H in retroviral-like particles (TY-elements) in *S. cerevisiae* impairs the ability of these elements to insert into the chromosome; probably as a result of inhibition of replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00069-21 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Mammalian Retrovirus Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.G. Levin	Research Biochemist	LMG:NICHD
Others:	W. Wu	Visiting Fellow	LMG:NICHD
	Z.Y. Yuan	Courtesy Contract	LMG:NICHD
	Y.-X. Feng	Guest Worker	LMG:NICHD
	J. Guo	Guest Worker	LMG:NICHD
	K. Post	Biologist	LMG:NICHD

COOPERATING UNITS *(if any)*

NICHD-LMG (R. Crouch); ABL Basic Research Program, NCI-FCRDC (A. Rein); CBER-DV (C. Marcus-Sekura); NCI-LEC (D. Hatfield); Mitsubishi Kasei Institute of Life Sciences, Tokyo (T. Uchida).

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Viral Gene Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.9	2.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulated expression of viral genetic information. Studies are being carried out on the functional relationship between the polymerase and RNase H domains of reverse transcriptase (RT), using purified murine leukemia virus (MuLV) RT proteins. These include wild-type RT and three mutants: (i) ΔH, which is missing the entire RNase H domain; (ii) a chimeric RT in which the MuLV RNase H domain is replaced with *E. coli* RNase H; and (iii) ΔSX, having a large deletion in a polymerase region corresponding to the "connection" subdomain in the p66 subunit of HIV RT. Our data show that each of these mutations, while occuring in only one domain, has profound effects on both polymerase and RNase H cleavage activities. We find, for example, that wild-type RT undergoes two types of RNase H cleavages. The major cleavage event is coordinated with DNA synthesis and occurs at a site in the RNA 14-18 nucleotides from the 3'-terminus of the primer; we refer to this cleavage as "3'-OH-dependent" since in this case, the 3'-OH of the primer is bound to the polymerase active site. Additional cleavages occurring closer to the 5'-end of the RNA are termed "3-OH-independer " i.e., the 3'-OH of the primer is no longer bound to the polymerase site and addition of dNTPs has little or no effect on the cleavage pattern. Interestingly, cleavages catalyzed by the ΔSX deletion mutant and the chimeric RT are exclusively 3'-OH-independent. Recent results evaluating the polymerase activity of the mutants indicate that the RTs with RNase H mutations cannot synthesize DNA in a processive manner. Experiments to determine whether these results reflect abnormal binding of primer/template will be performed. Future efforts will also include construction and analysis of other mutant RTs. In other work on translational control of viral gene expression, we have previously identified the *cis*-acting signal responsible for readthrough suppression of the UAG codon at the MuLV *gag-pol* junction. We are currently investigating the effect of this signal on normal translation of a transcript containing the glutamine codon CAG instead of UAG. Experiments are being carried out with infected cells and with an in vitro translation system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01002-11 LMG

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression During Embryonic Development of Xenopus Laevis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I.B. Dawid	Head	(All Personnel Listed LMG:NICHD)		
Others:	A. Karavanov	Vis. Scientist	M. Rebagliati	NRC Fellow	
	M. Taira	Vis. Scientist	M. O'Connell	NRC Fellow	
	R. Toyama	Vis. Associate	M. Rebbert	Chemist	
	J-P.Saint-Jeannet	Vis. Fellow	P. Curtiss	Biologist	
	H. Otani	Vis. Fellow	M. Bowker	Sum. IRTA	
	P. Good	IRTA Fellow	M. Kimura	Volunteer	
	C. Jungwirth	Courtesy			

COOPERATING UNITS *(if any)*
K. Geering and B. Rossier, University of Lausanne
W. Hayes, LDN, NICHD
H. Westphal, LMGD, NICHD

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
11.5	9.3	2.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms in embryogenesis of the amphibian Xenopus laevis and the zebrafish have been studied. The mRNA for the mesoderm inducer activin is absent from oocytes but abundant in follicle cells. Since activin protein occurs in the embryo, activin may be imported into the oocyte from the follicular layer. Inducing factors like activin elicit the expression of regulatory genes in specific patterns. The LIM class homeobox gene Xlim-1 is regulated by activin and retinoic acid and is expressed in three lineages: (1) The dorsal mesoderm during gastrulation, an area know as Spemann's organizer. Expression in this area is responsive to activin. (2) The pronephros. (3) Specific regions of the CNS. Expression in regions 2 and 3 is sensitive to RA, and depends on ectoderm-mesoderm interactions during and after gastrulation. The related genes Xlim-2 and Xlim-3 have been isolated and characterized; Xlim-3 displays a highly restrictive pattern in neural and neuroendocrine tissues. LIM genes have been isolated from the zebrafish and, in collaboration with H. Westphal, from the mouse; they are highly homologous in sequence and expression pattern to the Xenopus genes.
Transcriptional regulation of the Xlim-1 gene has been studied by injecting fusion constructs into the embryo. Animal explants derived from such embryos expressed the fusion construct very weakly unless stimulated by activin. Thus, the normal activin responsiveness of the Xlim-1 gene has been reconstructed, allowing the study of the putative activin response element of this gene. Studies on the formation of the nervous system focused on two projects. First, the role of RNA-binding proteins was studied by continuing the analysis of the nrp-1 gene and by isolating additional nervous system-specific genes that encode RNA binding proteins. Second, the relative importance of planar as opposed to transverse signals in neural induction was tested. Results obtained with embryos in which gastrulation movements were inhibited suggest that transverse induction is the dominant form of signaling in the formation of the nervous system.

024

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01004-10 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Amino Acid and Nucleotide Biosynthesis in Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A.G. Hinnebusch, Research Microbiologist, (All listed personnel LMG:NICHD)

C. Grant	Visiting Fellow	M. Marton	NRC Fellow	G. Pavitt	Guest Researcher
C. Vasquez	Visiting Fellow	P. Romano	IRTA Fellow	M. Garcia	" "
W. Yang	Visiting Assoc.	R. Rolfes	IRTA Fellow		
C. Moehle	Sen. Staff Fellow	J. Bushman	Pre-IRTA Fellow		
T. Dever	NRC Fellow	B. Jackson	Biologist		
C. Drysdale	NRC Fellow	N. Williams	Guest Researcher		
		E. Duenas-Santero	Guest Researcher		

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Genetics of Lower Eukaryotes

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
10.44	9.44	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

GCN4 is a transcriptional activator in S. cerevisiae that is regulated at the translational level by upstream open reading frames (uORFs) in the GCN4 mRNA leader. In amino acid-starved cells, ribosomes translate uORF1, bypass the remaining uORFs 2-4, and reinitiate at GCN4 instead because they fail to bind the eIF-2(initiation factor-2)•GTP•$tRNA^{Met}_i$ ternary complex. The level of ternary complexes is reduced in starved cells by phosphorylation of the α subunit of eIF-2 by GCN2. In mammalian cells, phosphorylated eIF-2 sequesters eIF-2B, the eIF-2-guanine nucleotide exchange factor. GCD1, GCD2, GCD6, GCD7 and GCN3, translational regulators of GCN4, were shown to be components of the eIF-2B in yeast. Mutations in eIF-2α, GCN3, GCD7 and GCD2 were isolated that suppress the toxic effects of eIF-2α hyperphosphorylation by $GCN2^c$ proteins, indicating that eIF-2 phosphorylation stimulates GCN4 translation by impairing eIF-2B. Deletions of GCN1 and GCN20 also suppress $GCN2^c$ alleles and the GCN1 and GCN20 proteins are required for GCN2-catalyzed phosphorylation of eIF-2α in vivo, probably functioning to couple GCN2 kinase activity to uncharged tRNA. Suppressors of $GCN2^c$ alleles affecting subunits of eIF-2 or eIF-2B overcome the toxic effects of expressing the mammalian eIF-2α kinases DAI and HRI in yeast, whereas GCN1 mutations only suppress $GCN2^c$ alleles, indicating that GCN1 is a positive regulator of GCN2 and not a negative regulator of an eIF-2α phosphatase. DAI is activated by double-stranded-RNA (dsRNA) during viral infections in mammalian cells, and translational control by DAI in yeast was shown to be dependent on amino acids in its regulatory domain that are conserved among dsRNA-binding proteins; thus, dsRNA-binding activates DAI kinase function in vivo. Purine starvation activates GCN2 in yeast and this response is required for wild-type purine biosynthesis and transcription of ADE8 under conditions of purine limitation. Genetic analysis revealed the existance of at least two non-overlapping activation domains in GCN4 that, to a first approximation, are functionally redundant. GCN4 proteins lacking one of these domains are toxic, reflecting the sequestration of a general transcription factor or repression of an essential gene. Chromosomal mutations were isolated that restore transcriptional activation by a gcn4 allele containing only an incomplete copy of one activation domain. We have also characterized mutations that alter transcriptional regulation of a ribosomal protein gene according to amino acid availability.

030

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01005-06 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cellular Proliferation and Diversity in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Kennison, Ph.D.	Senior Staff Fellow	LMG:NICHD
Others:	A. Felsenfeld, Ph.D.	Senior Staff Fellow	LMG:NICHD
	M. Vásquez, Ph.D.	Visiting Fellow	LMG:NICHD
	B. Brizuela	Bio. Lab. Tech	LMG:NICHD
	T. Brown	SIS	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. J. Tamkun, Department of Biology, University of California, Santa Cruz
Dr. R. Tjian, Dept. of Molecular and Cell Biology, Univ. of California, Berkeley
Dr. D.-H. Huang, Institute of Molecular Biology, Academia Sinica, Taiwan

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
4.6	3.0	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes involved in the determination and differentiation of adult tissues in *Drosophila melanogaster* have been identified by genetic interactions with homeotic and segmentation genes already known to be required in these tissues. Many new genes required for transcriptional activation of developmental genes have been identified. One of these new genes, *brahma*, encodes a large nuclear protein conserved from yeast to man. *brahma* mutations have in turn been used to identify other interacting genes required for transcriptional activation. The *osa* gene, which shows allele-specific interactions with *brahma* mutations, has been cloned by transposon tagging. The allele-specific interactions between *brahma* and *osa* suggests that the proteins interact directly in transcriptional activation. Genetic interactions with *brahma* have also been identified for the *trithorax*, *ash1*, and *kohtalo* genes.

The RNA polymerase II transcription factor TFIID is a complex of at least eight proteins. Chromosomal deficiencies that remove the genes encoding the 60 and 110 kDa polypeptides of TFIID have been identified. To isolate mutants for these two proteins, over ninety essential mutations have been generated in each chromosomal region. These mutations represent an average of four mutations per gene in each region. The wild-type genes have been reintroduced into transgenic strains by P-element-mediated transformation to determine which mutations affect the 60 and 110 kDa polypeptides.

Only one gene required for post-transcriptional regulation of homeotic genes has been identified. This is the *kismet* gene. *kismet* encodes a family of novel nuclear proteins, only one of which appears to be essential. The association of *kismet* proteins with specific sites on polytene chromosomes suggests that *kismet* may interact with the homeotic proteins in regulating their target genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01006-05

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-Nucleic Acid Interactions in Vertebrate Embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. Sargent Research Microbiologist (All listed are LMG:NICHD)

Others: P. Mathers NRC Biotech. Assoc. J. Franklin IRTA Fellow
R. Winning Visiting Fellow L. Shea Biologist
M. Morasso IRTA Fellow
K. Deonauth Pre-IRTA

COOPERATING UNITS *(if any)*

Milan Jamrich, M. Dirksen; CBER, FDA

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Vertebrate Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.75	3.75	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general aim of this project is to identify, characterize and elucidate the function of protein factors that regulate gene expression in vertebrate embryos. This is being approached in several ways. One strategy has been to identify transcription factors that activate specific genes in the early embryo. Initially, the focus of this approach was an epidermal keratin gene, XK81A1, expressed in the Xenopus laevis embryo. Analysis of cis-regulatory elements in this gene lead to the discovery that a Xenopus homolog of a mammalian transcription factor, AP-2 (XAP-2) plays an important role in keratin regulation, and is probably involved in other aspects of epidermal differentiation, both in frogs and in mammals. This work has continued, and is currently aimed at the regulation of the XAP-2 gene, itself a marker for early ectoderm. A parallel project is aimed at the regulation of XD112, an epidermis-specific homeodomain gene. The cis-regulatory DNA for this gene has been mapped to about 900 bp of 5' flanking sequence. This element drives epidermis-specific expression in both Xenopus embryos and in primary murine keratinocytes induced to differentiate in vitro.

A second strategy is directed at signal transduction mechanisms in cranial neural crest, a tissue induced to differentiate into a variety of cell types critical in formation of the vertebrate head. PCR-based techniques have been used to clone neural crest-derived cDNAs encoding protein kinases, a number of which have been obtained, falling into several categories. Most attention has been directed at a receptor tyrosine kinase in the Eph family, christened Pagliaccio (Pag), which is expressed in a localized pattern in brain and head structures.

The PCR has also been used to clone members of the cadherin family of cell-cell adhesion molecules from embryonic neural tube of the zebrafish (Brachydanio rerio). Cadherins are thought to play a role in neural crest migration and differentiation. This hypothesis will be tested by engineering transgenic fish in which the expression of specific cadherins can be experimentally altered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01007-04 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure, Function and Genetics of Nucleic Acid-Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01008-04

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Protein-Nucleic Acid Interactions in Drosophila

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

Susan R. Haynes	Expert	LMG, NICHD
Virginia M. Heatwole	IRTA Fellow	LMG, NICHD
David T. Stolow	NRC Research Associate	LMG, NICHD
Monica T. Monroe	Biologist	LMG, NICHD

COOPERATING UNITS (if any)

Ann Beyer, Department of Microbiology, University of Virginia Medical School
Gilles Thomas, Laboratory of Tumor Genetics, Institut Curie

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand the functions of Drosophila RNA binding proteins, particularly those related to the vertebrate A/B group hnRNP proteins, a major class of nuclear proteins that bind premessenger RNA. Biochemical studies of vertebrate hnRNP proteins suggest that they are involved in packaging and splicing nascent transcripts. We are using genetic and molecular biology techniques to study five Drosophila proteins that have similar RNA binding domains, two of which, Hrb98DE and Hrb87F, are hnRNP proteins. A common feature of at least three of the proteins is that they are required for male fertility.

Previously, we isolated a small deletion that removes the entire coding region of the Hrb87F gene. This mutation does not affect viability, but homozygous males are almost completely sterile. Recent experiments have shown that the deletion also removes a second gene, provisionally called BP1. The BP1 gene encodes a protein with two RNA binding domains that are similar to those of the Hrb proteins. The C-terminal domain is distinctive, and is unrelated to the other proteins. BP1 is expressed predominantly in pupae and adult males. Experiments are in progress to determine which gene is responsible for the male sterile phenotype and to analyze its function in spermatogenesis.

Mutations in the Rb97D gene, another RNA binding protein, also lead to male sterility. Rb97D is expressed in the testes and several other tissues during development, but appears to be required only during spermatogenesis, since the only obvious phenotype of a null mutation is male sterility.

EWS and TLS are human proteins that are disrupted in translocations associated with several sarcomas. A Drosophila homolog of these proteins is encoded by the p19 cDNA clone. The p19 protein has several glycine-rich regions and a domain that weakly resembles the RNA binding domains of the Hrb proteins. We have demonstrated that a polypeptide consisting of just the putative RNA binding domain can be cross-linked to RNA in vitro using UV light. Genetic characterization of this locus and further biochemical analyses of the protein are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01009-01 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation and Function of Genetic Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.L. Levin	Senior Staff Fellow	LMG:NICHD
Others:	M.J. Benedik	IPA Sabbatical Visitor	LMG:NICHD
	E.C. Sweeney	Bio Lab Tech	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. Jef Boeke, Johns Hopkins University, Baltimore, Maryland
Dr. Sandra Martin, University of Colorado Health Sciences Center, Denver
Dr. Carl Merril and Dr. Bill Walace, NIMH, St. Elizabeth's Hospital

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Yeast Molecular Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.09	1.17	.92

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our research program focuses on the mechanisms of retroelement action. The most medically important class of these elements are the retroviruses, examples of these are responsible for AIDS as well as several types of tumor formation. Our approach to understanding the complex interactions between the element and its host is to study retrotransposons, a family of elements that are closely related to retroviruses. A significant advantage of studying retrotransposons is they exist in hosts such as yeast that can be studied using sophisticated molecular genetic techniques. The specific element we have chosen to study is the retrotransposon Tf1 of the fission yeast Schizosaccharomyces pombe. We have previously shown that Tf1 has several structural similarities to retroviruses including two long terminal repeats (LTRs) and coding sequences with significant homology to retroviral protease (PR), reverse transcriptase (RT), and integrase (IN). More importantly, we have developed an in vivo assay for transposition that we used to shown that Tf1 is functional and can transpose by integrating a new copy of itself into an unrelated section of the genome. Results from work done during our first nine months at NIH include significant improvements made to the in vivo transposition assay. The assay depends on the overexpression of Tf1 transcripts containing a selectable marker that is reverse transcribed during transposition and inserted into the host genome. By modifying conditions we increased our previously reported transposition frequency of 0.5%/(11 cell generations) 40-fold to 18%. This improvement allowed us to develop a new transposition assay that is now being used to screen large numbers of cells for mutations that alter the transposition frequency. Our biochemical analysis of the transposon proteins has included the use of immunoprecipitation and in vivo protein labeling to determine the order and identity of the protease cleavage events of the 150 kDa precursor protein that results in the production of the mature Tf1 proteins.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD-00071-21-LMGD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene and Transgene Regulation in the Developing Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. Westphal, M.D., Chief, LMGD, NICHD
S. Bertuzzi, Ph.D., GW
J. Drago, M.D., Ph.D., GW
T. Fujii, M.D., Ph.D., VF
A. Grinberg, D.V.M., Veterinarian
M.L. Lakso, Ph.D. VA
E. Lee, D.V.M., Veterinarian
P.E. Love, M.D., Ph.D., CA/CO
B. Mosinger, Jr., M.D., Ph.D.,VA
T. Nakamura, M.D., Ph.D., VA
J. Pichel, Ph.D., VF
F.D. Porter, M.D. Ph.D. SSF
H. Sheng, M.D., Ph.D., VA
A. Zhadanov, Ph.D., VF

COOPERATING UNITS *(if any)*

EIB, DCBDC, NCI; SDB, LMG, NICHD, NIH.

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Section on Transgene Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
15.3	11.3	4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lens oncogenesis and differentiation. We generated mice that carry copies of a dormant transgene encoding the SV40 tumor antigens. The transgenes are specifically targeted to the lens and contain features that render their expression dependent on the action of Cre, a site-specific bacteriophage DNA recombinase. Timing of oncogene activation was controlled by making Cre available either prior to, or coincident with, the onset of primary fiber differentiation in the embryonic lens vesicle. Early expression of Cre resulted in oncogene activation in undifferentiated lens epithelial cells that rapidly proliferated inside the lens capsule. By contrast, when Cre accumulation was delayed to coincide with the onset of primary lens fiber differentiation, SV40 oncogenes were activated in cells that had begun to elongate and to accumulate lens-specific crystallins. During subsequent proliferation inside the lens capsule, transformed progeny cells maintained the profile of fiber differentiation that their parent cells had acquired at the time of oncogenic conversion. Our findings show that the differentiated state of the primary target cell is an important parameter of subsequent lens oncogenesis, and that an intact lens capsule can restrict invasive neoplastic growth. **The analysis of lim gene function** has become a new focus of research. Lim genes encode part of a network of functions involved in pattern formation and cell specification during metazoan development. Their intricate regulatory potential is indicated by the fact that they contain three adjacent regulatory regions, two lim domains and one homeo domain. We have begun our work on the lim genes by cloning and sequencing lim1, a homolog of the Xenopus laevis Xlim1 gene. The gene has been extremely well conserved during evolution, Transcripts are detected in the developing excretory system and in the central nervous system. **Role of the Zeta-family signaling molecules in T cell development.** The Zeta-family dimers are a group of structurally and functionally related proteins that are expressed as subunits of the T-Cell antigen receptor complex or as subunits of certain Fc receptors. In both contexts, they facilitate ligand-mediated signal transduction by coupling the surface receptor to intracellular signaling pathways. We have begun to analyze the role of the Zeta-family dimers in T-cells ontogeny by: 1) generating transgenic mice that overexpress each of these molecules or the overexpress mutated forms of Zeta, and 2) generating mice that lack Zeta, the predominant member of this group, by homologous-recombination. Our results suggest that the Zeta family dimers can perform complementary functions in development but that critical thymocyte signaling pathways are differentially responsive to these signaling molecules.

1

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-01802-05-LMGD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Genetic Basis of Mammalian Kidney Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gregory R. Dressler, Ph.D., LMGD, Head, Unit of Molecular Embryology
Uwe W. Rothenpieler, M.D., Visiting Associate, LMGD/NICHD
Larry. T. Patterson, M.D., IRTA Fellow, LMGD/NICHD
Gabriella Ryan, Ph.D., IRTA Fellow, LMGD/NICHD
Dawn E. Phelps, M.S., Pre-IRTA Fellow, LMGD/NICHD

COOPERATING UNITS *(if any)*

Urologic Oncology Section, Surgery Branch, NCI (J. Gnarra); The Wistar Institute, Philadelphia, PA (F.J. Rauscher)

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Unit of Molecular Embryology

INSTITUTE AND LOCATION

NICHD, NIH, 9000 Rockville, Pike, Bethesda, MD 20892

TOTAL STAFF YEARS.	PROFESSIONAL:	OTHER
4 years	3 years	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mammalian kidney is used as a model to study the genetic basis of development and oncogenesis in a complex multicellular tissue. Current studies continue to focus on the role of transcription factors in early kidney development and their regulation by potential tumor suppressor genes. The objectives are to understand how developmental regulatory genes can contribute to oncogenic transformation.

A. Development. The Pax-2 gene is activated early after kidney induction, in mesenchymal cells destined to become the renal epithelium. Using in vitro embryonic kidney cultures and antisense oligonucleotides against the Pax-2 gene, we have shown a clear requirement for this transcription factor in the transition of the kidney mesenchyme to epithelium. Furthermore, the requirement for Pax-2 is transient, as the terminally differentiating proximal and distal epithelial cells suppress Pax-2 expression. Persistent expression of Pax-2 in transgenic mice results in severe kidney abnormalities similar to human congenital nephrotic syndrome.

B. Oncogenesis. The suppression of Pax-2 during kidney development is mediated, in part, by the Wilms' tumor suppressor gene, WT1. There is a direct correlation between decreasing levels of Pax-2 protein and increasing levels of WT1 in the developing nephron. Furthermore, WT1 binds at least three sites in the Pax-2 5' regulatory sequences and can repress transcription in vitro. Pax-2 is expressed in Wilms' tumor, an embryonic tumor derived from mesenchymal blastema or undifferentiated epithelium. Pax-2 expression can also be detected in renal cell carcinoma from adult patients. This may be due to reactivation of Pax-2, or hyperproliferation of a renal stem cell population that has never repressed the Pax-2 gene. in any event, cell lines derived from renal cell carcinomas can be growth inhibited with Pax-2 antisense oligonucleotides, suggesting that activation of Pax-2 is a necessary determinant for oncogenesis.

C. Biochemistry. In order to understand the function of the Pax family of transcription factors, identifying the potential target genes is mandatory. using Pax-2 specific antibodies, DNA-protein complexes were precipitated from intact chromatin and the DNA binding sequences cloned. DNA footprinting and methylation protection experiments clearly demonstrate Pax-2 binding to these genomic loci. These genomic tags can be used to identify larger genomic clones that may contain potential target genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-01801-04-LMGD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

The Molecular Analysis of Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

K.A. Mahon, Ph.D., Expert
D.E. Feltner, M.D. Guest Worker
L. Gleizer, Ph.D, Visiting Fellow
E. Hermesz, Visiting Associate
M.D.Johnson, M.D., Medical Staff Fellow
M.A. O'Reilly, Ph.D., NRC Fellow
G.W. Robinson, Ph.D., NRC Senior Associate

COOPERATING UNITS *(if any)*

CBER, FDA (M. Jamrich), LP, NCI (S. Mackem), LMG (T.Sargent), MGL, NCI-Frederick Cancer Research Facility (N. Jenkins, N. Copeland), U. of Mich. (S.Camper), Hosp. Sick Children, Toronto (L.-C. Tsui).

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Unit on Developmental Gene Regulation

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS.	PROFESSIONAL:	OTHER
5.2	5.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This group has focused on the conserved homeobox DNA binding domain as a means to gain insight into the molecular basis of early embryonic development in mammals. Homeobox genes occupy key positions in the regulatory gene hierarchy responsible for establishing the embryonic body plan in *Drosophila*, and are thought to play analogous roles in the development of higher vertebrates. Several novel homeobox genes have been previously isolated by the laboratory that are expressed in early embryogenesis. Current research has concentrated on the *Rpx* gene, which possesses the most anterior-restricted expression domain of all known homeobox genes. Transcripts are seen very early in the cephalic neural plate, and become progressively restricted to Rathke's pouch, the rudiment of the anterior pituitary, indicating a potential role for the *Rpx* gene in the development of this organ. Experiments to determine how this gene is regulated in the embryo and how it functions in development are in progress. Chimeric fusion genes consisting of varying amounts of the 5' flanking sequence of the gene linked to the bacterial beta-galactosidase (*lacZ*) reporter gene have been used to generate transgenic mice. Analysis of fusion gene expression in transgenic embryos indicates that all of the information sufficient for proper temporal and spatial expression resides within 1 kb of upstream sequence. The sequence of this region is characterized by the presence of several motifs found in the regulatory regions of other genes expressed in the anterior pituitary, at least one of which is conserved between the mouse and frog homologs of the *Rpx* gene. More precise deletion or mutation of these regions is underway in order to more accurately dissect the elements necessary for proper expression. Determination of elements specifically required for *Rpx* expression will potentially lead to the identification of genes that occupy earlier positions in a regulatory hierarchy at work in the early embryo. Experiments designed to investigate the function of *Rpx* include generating gain-of-function and loss-of-function models by ectopically expressing *Rpx* gene products in transgenic mice and by mutating the gene through homologous recombination in embryonic stem cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 08719-13 LMGR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development and Uses of Eukaryotic Vectors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Bruce H. Howard	Chief	LMGR,NICHD
Other:	M.L. Avantaggiati	Visiting Fellow	LMGR,NICHD
	C. Driscoll	Biologist	LMGR,NICHD
	V. Ogryzko	Visiting Fellow	LMGR,NICHD
	E. Englander	Visiting Associate	LMGR,NICHD
	G. Humphrey	Sr. Staff Fellow	LMGR,NICHD
	V. Russanova	Visiting Fellow	LMGR,NICHD
	K. Engleka	NRC Bio Tech	LMGR,NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Human Cell Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	6	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of research in the Laboratory of Molecular Growth Regulation is on control of cell proliferation in mammalian cells. Of particular interest are interrelationships between growth control and basic processes such as cellular differentiation and aging. Using recently developed gene transfer technology in conjunction with the standard array of recombinant DNA methodologies, several related questions are being investigated: i) What are the molecular mechanisms that control cellular senescence in normal human cells? ii) Why do rodent cells become immortalized at a far higher frequency than human or other primate cells? iii) What roles do cellular anti-oncogenes encoding p53 and retinoblastoma gene products have in cellular quiescence and senescence? and iv) Do interspersed repetitive sequences, which comprise 5-10% or more of mammalian genomes, represent unconventional but fundamentally important elements in the control of differentiation, tumor suppression and cellular senescence.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00412-06 LMGR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Regulation of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Richard J. Maraia	Senior Research Investigator	LMGR, NICHD
Other:	D.-Y. Chang	NRC BioTech Fellow	LMGR, NICHD
	J. Sarrowa	IRTA	LMGR, NICHD
	T. Howard	Chemist	LMGR, NICHD

COOPERATING UNITS (if any)

Department of Pathology, Texas Children's Hospital (Gretchen Darlington)
Department of Microbiology/Immunology, Duke Univ. Medical Center (Jack Keene)
Section on Molecular and Cellular Biology, Univ. of California, Davis (Carl Schmid)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Human Cell Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research on repetitive genetic elements of humans and other mammals was continued. Alu sequences are derived from small transposed elements endogenous to the human genome. Nearly one million dispersed copies constitute 5% of the genetic material. Alu mobility has caused genetic variability and multiple heritable disorders in humans. The regulated expression of Alu and its mouse homologue B1 into RNA was studied. Germline and fetal-specific expression suggests their involvement in normal development. Examination of mechanisms of B1 and Alu RNA expression continued to yield novel results and insight into their complex regulation and propensity for transposition.

1. Advances in transcriptional regulation were made. A transcription termination/reinitiation factor for RNA polymerase III was characterized. Methods to isolate native transcription complexes by affinity chromotography and to improve the study of transcriptional regulation were developed.

2. Protein factors that interact with these repetitive DNA elements to regulate their expression, as well as proteins that modulate the metabolism of their RNA products were characterized.

3. An Alu RNA binding protein was purified to apparent homogeneity and a partial amino acid sequence was determined. From this information a cDNA was isolated and characterized.

4. Sequences that encode several small RNAs were mapped to single human chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01310-06 LMGR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Gene Regulation of the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: K. Ozato Head, SMGI LMGR, NICHD

See Attached

COOPERATING UNITS (if any)

Unit on Molecular Mechanisms of Reproductiion (Dr. James Segars)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
12.7	10.25	2.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This section has been studying gene regulation in the developing immune system. Our focus has been transcriptional regulation of genes mediated by retinoids/ vitamins and interferons (IFNs). These agents exert regulatory activities partly through transcription factors called RXRß and ICSBP that we have previously isolated. RXRß is a member of the nuclear hormone receptor superfamily and heterodimerizes with many other members of the superfamily. We have shown that RXRß heterodimerizes with retinoic acid (RA) receptor ß, and plays a key role in RA induction of MHC class I genes in human embryonal carcinoma (EC) cells. To further elucidate the role of RXRß heterodimers in vivo, several dominant negative mutants of RXRß have been constructed and their activities examined in P19 EC cells. Our data show that a mutant lacking the DNA binding domain inhibits RA induction of several genes, by inhibiting the function of RXR/RAR heterodimers formed in vivo. We found that this inhibition also leads to an altered growth regulation in the EC cells. By genomic footprinting analysis, it was found that the RA responsive element of an RA inducible gene is not occupied prior to RA treatment in undifferentiated EC cells, but the occupancy is promptly induced after RA treatment. This is the first demonstration that RXR heterodimers bind to cognate DNA only after RA treatment in vivo, which is in contrast to the RA independent DNA binding in vitro. IFNs are cytokines produced in many types of cells, and are being used for treatment of cancers and viral infections. IFNs' biological activities are realized through the action of the IRF family, to which ICSBP belongs to. We showed that ICSBP represses IFN induction of many IFN regulated genes, which is attributed to the interaction of ICSBP and other members of the family. We found that ICSBP directly binds to IRF-1 and IRF-2, which enhances binding of ICSBP to cognate DNA. This association can be detected in vitro, but occurs also in vivo, and in vitro. In the coming year we will focus on mechanistic analysis of how these factors acts. Interactions of RXRß and IRF family members with basal transcription factors will be studied for both physical and functional interactions. The recently developed in vitro transcription assay involving recombinant RXRß will be used for functional analysis.

020

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00410-07 LTPB

PERIOD COVERED

October 1, 1992 to December 31, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism in Children with Glycogen Storage Disease, Type I

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: James B. Sidbury Scientist Emeritus LTPB, NICHD

COOPERATING UNITS (if any)

Human Genetics Branch, NICHD

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transferred to LTPB, but the project is presently inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-18 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Characterization of Macromolecular and Cellular Particles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	A. Chrambach	Head	SMA, LTPB, NICHD
Others:	M. Garner	Expert	SMA, LTPB, NICHD
	D. Wheeler	IRTA	SMA, LTPB, NICHD
	Z. Buzas	Courtesy Associate	SMA, LTPB, NICHD

COOPERATING UNITS (if any)

Agricultural Biotech. Ctr., Institute for Molec. Genetics, Hungary (Dr. L. Orban)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DNA-protein complexes are the biologically relevant DNA species par excellence. Thus, while previous electrophoretic DNA characterization has dealt with conformational analysis of free DNA, this year's project aimed at such analysis of DNA-protein complexes. Two classical, well-studied complexes provided suitable models for such work: The nucleosome and the E.coli-DNA-CAP complex. It was found that in contrast with the free nucleosomal or standard linear DNA which are stretched in proportion to gel concentration, the nucleosome maintains its globular conformation, brought about by the wrapping of 1.5 superhelical turns around histone octomer, independently of gel concentration, i.e. the conformation of the complex in a gel is that of a protein, not of DNA. Electrophoretic conformational analysis of DNA-protein complexes was also applied to the problem of distinguishing peripherally and centrally bound CAP promoter on a 146 bp DNA fragment by transverse pore gradient gel electrophoresis. A difference in deformability by the gel was found. It is due to differences in curvature of the DNA fragment carrying the specific binding site on which the well-known DNA bending by CAP is superimposed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00171-17 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrophoretic Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	M.M. Garner	Expert	LTPB, NICHD
	D. Wheeler	IRTA	LTPB, NICHD
	T. Guszczynski	Visiting Fellow	LTPB, NICHD
	Z. Buzas	Courtesy Associate	LTPB, NICHD
	D. Tietz	Adj. Scientist	LTPB, NICHD
	A. Aldroubi	Senior Staff Fellow	NCRR, NIH
	R. Costello	Collaborator	Clinical Pathology, CC

COOPERATING UNITS (if any)

Agricultural Biotech. Ctr., Institute for Molec. Genetics, Hungary (A. Zsolnai, L. Orban); Institute of Analytical Chemistry, Brno, Czech Republic (P. Gebauer, P. Bocek)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The design of effective separation systems for bent and other forms of conformationally "abnormal" DNA requires an understanding of the causes of gel retardation. We have sought such understanding by computer simulations of the passage of variously shaped objects through arrays of obstacles, i.e. a highly simplified model of a gel. We have also initiated a study in polymer solution - and in "Mutation Detection" - electrophoresis (MDE) to correlate polymer structure with retardation. 1) Computer modeling of the passage of variously shaped particles through arrays of circular obstacles demonstrated progressive retardation of rod-shaped particles with bending; a rate of passage of circular particles greater than that of rods at low densities of obstacles and less than that of rods at higher densities, with complete arrest at a sufficient obstacle density; progressive rates of passage upon increase in flexibility of the particle; decreasing rates of passage with increasing axial ratio or curvature and, most dramatically, increasing length of curved surface. These rates of passage correlate with the mobilities of the analogous DNA conformers. 2) Using the same technique of computer modeling, the applicability of the Ogston model to prolate ellipsoid particles was demonstrated. Application to non-spherical particles requires prefactors which were defined for prolate ellipsoids and can potentially be defined for other non-spherical particle shapes. 3) DNA electrophoresis conducted on agarose gels submerged under an immiscible solvent followed by ethidium bromide staining provides relative fluorescence intensities that are independent of DNA size in the range investigated (up to 2 kb) while those derived from the conventional "submarine" technique fall off sharply with DNA size. 4) The separation of standard from heteroduplex DNA on a transverse MDE gel is independent of gel concentration. The finding allows for shortened MDE gel analysis, and suggests a pseudo-charge separation mechanism for "kinked" DNA. 5) Intrinsic viscosity values and retardation coefficients for polystyrene sulfate size standards were determined in solutions of polyacrylamide, dextran, polyvinyl alcohol, polyethylene glycol, hydroxyethyl cellulose and polyvinylpyrrolidone of various sizes. An attempt is being made to correlate particle size and effective polymer size for separation within a given particle size range. These combined studies have shown that a) the "abnormal" retardation of bent and circular small DNA is likely to be purely due to geometric properties and is accentuated through molecular flexibility. b) Delicate conformational changes which are not expressed by the shape of the electrophoretic pattern in a gel concentration gradient ("kinks") are responsible for resolution in MDE. c) Molecular sieving of large macromolecules seem most effective at low polymer concentrations and commensurate end-to-end distances of polymers in solution. These findings provide elements for the rational design of polymer architecture for effective molecular sieving.

5

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-11 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Applications of Metal Stable Isotopes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	A. Yergey	Head	SMAMS, LTPB, NICHD
Others:	N. Vieira	Biologist	SMAMS, LTPB, NICHD
	R. Goans	Senior Staff Fellow	SMAMS, LTPB, NICHD

COOPERATING UNITS (if any)

PSL, DCRT (G. Weiss); Human Genetics Branch, NICHD (J. Marini); Child Nutrition Research Center, Houston, TX (S. Abrams); Nemours Children's Clinic, Jacksonville, FL (N. Mauras); The Mayo Clinic, Rochester, MN (L. Fitzpatrick, B.L. Riggs)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. The study of calcium absorption and distribution in hypogonadal adolescent males has been extended to a group of six subjects. All subjects were studied at baseline and subsequently after a period of treatment that consisted of 2 injections each of 100mg testosterone enanthate spaced 3 weeks apart. Results show an average increase in calcium absorption of 61% coupled with a 108% increase in whole body calcium balance. These results were consistent with similar observations of increased protein synthesis rate determined from measurements of leucine metabolism as measured by glutamate oxidation and keto-isocaproic acid production from leucine as measured by our collaborators. 2. Short-term calcium kinetics measurements from 76 adolescent and adult women have been used to develop a clinical test for bone affinity for calcium. The test was developed by modeling short-term plasma tracer dilution kinetics using a power function clearance model. This model of calcium clearance gives a simple and reliable estimate of early bone accretion in the first three hours after injection of calcium isotope. The test is capable of differentiating accretion in adults from that in Tanner 1-3 adolescents at $p < 0.001$ using the Mann-Whitney Rank Sum Test. The model has been applied to the analysis of osteopenia in adolescent women with severe anorexia nervosa. The model has documented an increase in bone accretion of 158% after nutritional stabilization in a 16 year-old subject. 3. Studies of the crystallization kinetics of hydroxyapatite and other calcium phosphates have been used to develop a novel model of bone mineralization that depends on the Ca/PO_4 ratio in salts. When the non-linear parameter of this equation is fit to crystallization kinetics or observed isotope dilution data from human studies we have found that: a) the fit in adult subjects is remarkably similar to that for the crystallization of hydroxyapatite b) the fit for data sets from children suggests the presence of Ca/PO_4 ratios consistent with more soluble phosphates that might be expected to occur in the more rapidly turning over bone of immature subjects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01401-11 LTPB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biological Applications of Liquid Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	A. Yergey	Head	SMAMS, LTPB, NICHD
Others:	K. O'Brien	IRTA Fellow	SMAMS, LTPB, NICHD
	X. Zhang	IRTA Fellow	SMAMS, LTPB, NICHD

COOPERATING UNITS (if any)
Developmental Endocrinology Branch, NICHD (F. Cassorla, K. Weiner, K. Oerter); Dept. of Nutrition, Univ. of Connecticut, Storrs, CT (L. Allen, M-L. Caldera)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
2.3	2.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. We have determined the effect of calcium deficiency on vitamin D kinetics using our previously developed thermospray methodology. We have determined that the half-life of 25OHD is about 50% shorter under situations of low calcium intake and negative calcium balance. On a 950 mg/day Ca intake, 25OHD half life was 9.5 days, comparable to our earlier results. On a low calcium intake, 200 mg/day, the half life was reduced to 4.9 days. These values are consistent with a faster conversion of 25OHD into the biologically active form of vitamin D that would serve to enhance absorption under conditions of low calcium intakes. 2. We have shown that the monohydrate of the $(C_6H_5)_4As^+$ cation formed under electrospray conditions is in equilibrium with the unhydrated ion. By use of the van't Hoff plot methodology, we have determined the enthalpy change for the addition of a single water to $(C_6H_5)_4As^+$. The value obtained, -12.6 kCal/mole, is comparable to the value obtained by other workers for the addition of a single water to the Cs^+ ion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01408-03 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stability and Specificity of DNA-Protein Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Garner	Expert	SMA, LTPB, NICHD
Others:	A. Chrambach	Head	SMA, LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Metabolism, NIDDK (D.C. Rau)
Laboratory of Molecular Biology, NCI (S. Adhya)
Center for Biologics Evaluation and Research, FDA (Gerald Marti)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The binding of a gene regulatory protein to DNA brings together two complementary surfaces, each of which interacts with water and ions in the solution. When the two macromolecules bind, some of these interactions with the milieu are lost, and new interactions with the complementary surface are formed. This exchange of interactions with water and ions for interactions with the other macromolecule makes the extent of binding very sensitive to solution conditions. In addition, DNA-mediated protein-protein interactions (e.g. DNA looping), are an important part of the biological activity of most transcriptional regulators. Varying the solution oncotic pressure (by adding high concentrations of an excluded solute) allows one to determine the effect of surface hydration of both protein and DNA on complex formation. This allows one to determine the total number of water molecules released in the binding reaction, which should be related to the amount of surface area of both protein and DNA occluded in the complex. Such insight into the hydration of both surfaces before and after complexation is important for understanding the sources of stability and specificity of a protein-DNA complex. In addition, condensed metaphase chromosomes from Chinese Hamster Embryo cells were subject to electrophoresis in liquid polymer solutions, in an attempt to perform both preparative and analytical separations of mammalian chromosomes. The ability to perform such separations will greatly facilitate the physical mapping of genes to specific chromosomes or chromosome regions.

2c

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01409-08 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Membrane Transport and Fusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. Zimmerberg	Chief	LTPB, NICHD
Others:	S. Sokoloff	Visiting Associate	LTPB, NICHD
	S. Chanturia	Visiting Associate	LTPB, NICHD
	I. Plonsky	Visiting Fellow	LTPB, NICHD
	T. Whalley	Visiting Fellow	LTPB, NICHD
	E. Suss-Toby	Visiting Fellow	LTPB, NICHD
	M. Cho	Biologist	LTPB, NICHD
	V. Ratinov	Adjunct Scientist	LTPB, NICHD

COOPERATING UNITS (if any)

Frumkin Institute, Moscow, Russia (Y. Chizmadjev); Israel Institute, Ramat Gan, Israel (D. Kaplan); UCL, London, (M. Whitaker)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
4	3 1/4	3/4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cells must merge membranes in order to mix aqueous spaces. We have made progress this year in developing new methods for the characterization of fusion pores, and the identification and characterization of eukaryotic fusion proteins. Using double whole-cell recording technique, junctional conductance was measured during baculovirus-mediated syncytia formation of insect cells. Fusion was triggered by low pH. Symmetrical currents in each cell of the pair measured the fusion pore conductance directly. It started as abrupt (0.1-0.2 ms rise-time) pore formation with a distribution of conductances centered at 1 nS, widened slowly in the time scale of tens of seconds and then increased abruptly. Our previous method, time-resolved admittance measurement, was performed to compare two methods: both gave similar results. Thus our previous methods of measuring fusion pores was confirmed. The new method will allow us to improve the time resolution of the fusion pore conductance by 1-2 orders of magnitude. A multiple-frequency, digital lock-in amplifier was implemented on a microcomputer to extend our measurements of fusion pores so as to calculate the fusion pore conductance more precisely in the presence of noise. To determine the proteins involved in fusion pore formation, thiol group sensitivity was studied. Amino-dextran derivatives of the reversible thiol reagent SPDP inhibit exocytosis inversely with size. This implies that the susceptible thiol groups are not buried deeply but are in a relatively exposed hydrophilic environment. Thiol-reactive proteins are involved in triggering fusion rather than downstream events, since no fusion is seen upon the removal of the SPDP block by DTT treatment. GTP-triggered fusion of rat liver microsomes was strongly inhibited by sodium metaperiodate. The restorative effect of DTT indicates that at least the reversible portion of the inhibition is caused by oxidation of protein vicinal thiol groups with the formation of disulfide bonds. Selective labeling of periodate-sensitive thiols revealed one major labeled polypeptide with M.W. of 15 kDa. The correlation between oxidation and reduction of the protein and inhibition and restoration of the microsomal fusion, respectively, suggests that this protein may be part of the GTP-dependent microsomal fusion machinery.

2c

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01413-03 LTPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Microscopic Analysis of Drug Induced Alterations in DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

Nicholas J. Rampino, Ph.D.

COOPERATING UNITS *(if any)*

LAB BRANCH

SECTION

INSTITUTE AND LOCATION

Laboratory of Theoretical and Physical Biology

TOTAL STAFF YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Transferred to the National Institute on Aging

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 14115-03 LTPB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Exocytotic Membrane Fusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	S. Vogel	Senior Staff Fellow	LTPB, NICHD
Others:	L. Chernomordik	Visiting Scientist	LTPB, NICHD
	M. Cho	Biologist	LTPB, NICHD
	T. Whalley	Visiting Fellow	LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS (if any)
Neurobiology, NINDS (M. Terasaki)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2 1/4	2	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used components of the sea urchin egg cortical reaction to study the mechanism of calcium triggered exocytosis. Using reversible inhibitors of membrane fusion we have shown that the molecular steps which respond to free calcium, and are involved in triggering membrane fusion, are distinct from subsequent steps that are involved in the actual membrane merger. Studies of the calcium dependence of exocytosis under conditions where we progressively destroy the fusion machinery with mild NEM treatment (under conditions where we still achieve 100% vesicle fusion) show a reduction in the rate of fusion as well as an increase in the concentration of calcium where we observe the onset of fusion. This is consistent with predictions based on a computer simulation which we have written to model the calcium dependence of exocytosis as a function of the number and apparent Kd of fusion proteins per granule. Our finding that isolated cortical granules can fuse with liposomes, argues that the proteins mediating membrane fusion reside on the exocytotic granules and do not require ancillary proteins on the plasma membrane for fusion. Comparative inhibition of exocytosis in vitro and in vivo reveal that short wavelength UV light inhibits exocytosis at steps prior and subsequent to membrane fusion and suggest that the UV inhibition prior to fusion can be reversed by components present in the intact egg. In most cells exocytosis is followed by endocytosis to maintain membrane homeostasis. We have developed assays to measure endocytosis in sea urchin eggs so that we can study the coupling between exocytosis and endocytosis. Our preliminary studies show that endocytosis can be triggered by sperm or calcium ionophores, and inhibition of exocytotic granule fusion forestalls endocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01416-01 LTPB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Tissue Imaging in Cell Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. Zimmerberg	Chief	LTPB, NICHD
Others:	L. Margolis	Courtesy Associate	LTPB, NICHD
	S. Glushakova	Courtesy Associate	LTPB, NICHD
	B. Baibakov	Courtesy Associate	LTPB, NICHD
	F. Suprynowicz	Senior Staff Fellow	LTPB, NICHD
	P. Blank	IRTA Fellow	LTPB, NICHD

COOPERATING UNITS (if any)
Duke University (S. Simon); NINDS (C. Collins); Rush University (S. Hollenberg)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

We have begun to study aspects of cell behavior in thick tissues: cell locomotion, cell proliferation and cell fusion. These fundamental biological processes in multicellular organisms have for many years been studied on cells isolated from tissue. However, in vivo, all these events are influenced by the complex, heterogeneous microenvironment of living tissue. We have developed a new approach to studying cell behavior which includes long-term culturing of blocks of unperturbed mouse and human tissues and confocal laser microscopy of individual cells inside these tissues. These newly developed techniques have been used to study tumor invasion of normal tissue. We have shown that the behavior of tumor cells is significantly affected by their surrounding normal tissue. An inhomogeneous tissue microenvironment results in nonuniform invasion of tumor cells which produce "streams" of cells following each other. This system provides an opportunity for direct observation of tumor invasion and raises the possibility of studying the effects of various drugs on this process.

Thick tissue imaging was also implemented in vivo to test the mechanism of septic shock. Resistance arterioles of the rat cremaster muscle were imaged and the response to catecholamines was measured. Septic animals showed impaired vasoconstriction compared to controls. Inhibiting nitric oxide synthase reversed this hyporesponsiveness. By imaging the vessels actually responsible for the decrease of TPR seen in sepsis, pathophysiologic hypotheses can thus be directly tested rather than extrapolated from model tissues such as aorta.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01501-02 LTPB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of Lipids in Membrane Rearrangements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	L. Chernomordik	Visiting Scientist	LTPB, NICHD
Others:	S. Vogel	Senior Staff Fellow	LTPB, NICHD
	A. Sokoloff	Visiting Associate	LTPB, NICHD
	E.A. Leikina	Visiting Associate	LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Membrane and Cellular Biophysics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2 1/4	1 3/4	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We study the mechanisms of biological membrane fusion, a ubiquitous process in cell biology. To elucidate the key intermediates of lipid bilayer rearrangements in the fusion reaction, we investigated the effects of membrane lipid composition on the rates of diverse fusion processes including Ca++-triggered cortical exocytosis and low pH-triggered syncytia formation of baculovirus infected insect cells. Lysolipids, naturally-occurring lipids, whether added exogenously or produced in situ by phospholipase A2 treatment, were found to arrest fusion reversibly at an "activated state" between fusion-triggering and membrane merging. In both experimental systems the specific fusion trigger (H+ or Ca++) was applied in the presence of inhibiting concentrations of short-chain lysophosphatidylcholine. Next the cells and granules were washed with buffers containing lysolipid but without a trigger. No fusion or lysis was observed at this stage. Removal of the lysolipid 5-10 min later by washing with buffers lacking the fusion-trigger resulted in the initiation of fusion. The extent of fusion was comparable with samples which were not treated with lysolipids. The "trigger-independent" stage of biological fusion, arrested by lysolipids, required trigger application to contacting membranes for its development. Our results suggest that i) while different fusion processes utilize different triggers, the pivotal step involving membrane merger is trigger independent and lipid sensitive, ii) fast exocytotic processes share a common mechanism for membrane merger with slow viral fusion, and, finally, iii) diverse biological fusion reactions proceed through highly bent membrane intermediates, "stalks", which cannot form when inverted-cone-shaped lysolipids are present in the contacting leaflets. It is also intriguing to speculate that membrane phospholipids, controlled by phospholipase activity, might regulate calcium-triggered exocytosis by integrating multiple calcium pulses into a coordinated exocytotic event.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00093-19 OSD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action of Nerve Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Guroff Head, SGF OSD, NICHD

Others: See attached

COOPERATING UNITS *(if any)*

See Attached

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Growth Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
10.00	8.50	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nerve growth factor (NGF) is the best characterized of the peptide growth factors acting on the nervous system, and is now recognized as a member of a family of growth factors, the neurotrophins, that supports a wide variety of neural cells. Initial studies on NGF demonstrated that it was required for the survival and differentiation of sympathetic and sensory neurons. It is now known, in addition, to influence the development of a wide variety of cell types, including certain neurons in the central nervous system, the chromaffin cells of the adrenal medulla, a number of tumor cells, and cells of the immune system, as well. The actions of NGF on these different cells are not necessarily the same, but they are all initiated by the binding of the peptide to specific, high-affinity receptors on the surface of the cell. The binding of NGF to its physiologically-responsive, tyrosine kinase receptor, now known to be the protein product of the trk proto-oncogene, initiates a number of intracellular actions that lead to alterations in the function of key proteins in the cell and to changes in the expression of specific genes. These changes in protein function and in gene expression are the mechanism by which NGF exerts its effects on the cells. These biochemical changes have been studied, in large measure, in PC12, a cell line derived from a tumor of the rat adrenal medulla. This cell line represents one of the most informative differentiating systems available. In the presence of NGF it stops dividing, elaborates neurites, becomes excitable and, to all intents and purposes, goes from a rapidly dividing chromaffin cell to a terminally differentiated sympathetic neuron in a matter of a few days. With this system it is possible to ask questions about how NGF acts, not only at the biochemical level, but also how it produces such global changes in the cell phenotype. Using this system it has been established that the actions of NGF are mediated, at least in part, by changes in the phosphorylation of key proteins in various compartments of the cell, including the nucleus. It is further known that these phosphorylative changes are accompanied by changes in the function of these proteins. One of the functional changes that may be part of the mechanism by which the cell survives is an NGF-induced change in the ability of the cell to take up calcium, since neuronal survival appears to be fostered by increased intracellular calcium. The ability of NGF to regulate cellular calcium may also be involved in the neuroprotective actions of the neurotrophins, since high levels of calcium produced by ischemia or hypoglycemia may contribute to the death of neurons after these cerebral insults. Another functional change that may be part of the mechanism by which the cell is instructed to stop dividing is an NGF-induced decrease in the number of mitogen receptors on the cell surface, since such receptors regulate the ability of mitogens to promote cell division. It is reasonable to expect that a detailed knowledge of how NGF acts in this system will illuminate the overall mechanism of neuronal differentiation and survival. This, in turn, could provide insights into disease states in which neurons either develop inappropriately or die prematurely.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01500-11 OSD

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on DNA Replication, Repair & Mutagenesis in Eukaryotic & Prokaryotic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: A.S. Levine, Head, SDRRM, OSD, NICHD

R. Woodgate	Sr. Staff Fellow	M. Takao	Visiting Fellow
M. Protic'	Sr. Staff Fellow	J. McDonald	IRTA Fellow
M. Carbone	Visiting Associate	C. Cicala	Visiting Fellow
E. Chumakov	Visiting Fellow	M. Avantagiatti	Visiting Fellow
O. Studitskaia	Visiting Fellow	J. Hauser	Microbiologist
V. Rapic' Otrin	IRTA Fellow	M. McLenigan	Chemist

COOPERATING UNITS *(if any)* LMG, NICHD (D. Ennis, S. Haynes); FDA (W. Koch, K. Chumakov, M. Moos, J. Beer); NCBI (J. Wootton); U.-MO (S. Alexander); NICHD, OSD (G. Guroff); U. di Chieti (A. Procopio); NCI (H. Pass); NW U. (K. Rundell); Freie U. (A. Graessmann); NIDDK (E. Ackerman); UNC (J. Griffith); Col. U. (W. Hendrickson); NIMR (S. Sedgwick)

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on DNA Replication, Repair, and Mutagenesis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
12.0	10.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the mechanism of **prokaryotic mutagenesis** have focused on the roles of the **RecA** and **UmuD, C**-like mutagenesis proteins (which facilitate bypass of lesions by DNA polymerase during replication of damaged DNA). Biochemical assays have revealed that UmuD, its cleavage product (UmuD'), and the functionally homologous, plasmid-encoded **MucA'** proteins physically interact with RecA. This interaction may provide a mechanism by which the Umu-like proteins are targeted to lesions in DNA. Two ***E. coli*** strains have been constructed that allow the identification of Umu phenotypes based upon a simple phenotypic complementation assay. These strains have facilitated the cloning of three new *umu*-like operons from **R-plasmids** R391, R446b and R471a. These tester strains were also utilized to identify several novel plasmid-encoded *umuC* mutants. In studies on **mammalian DNA repair,** we determined the subcellular distribution and regulation by **UV** light of a 127 kDa protein component of a primate **UV-damaged DNA-binding** (UV-DDB) complex. Structural homologs of this **UV-DDB** protein were identified in slime mold and rice, and a partial cDNA of a Drosophila homolog was isolated. New evidence supports an important role for the UV-DDB complex in mammalian DNA damage-recognition and repair: One additional **Xeroderma Pigmentosum** patient ("XP variant") with a defect in UV-DDB activity was identified (4 patients are now known to lack this damage-recognition activity); the recovery of UV-DDB activity is delayed in UV-irradiated cells from XP groups A, D, and C patients, which correlates well with their DNA repair-deficiency; and cells from mammalian tissues that do not normally express UV-DDB activity are more UV- sensitive than their clonal isolates which have regained the binding activity. In studies on **SV40** as a model eukaryotic replicon, we focused on the viral **small t-antigen**. In a purified ***in vitro* SV40 DNA replication** system, we found that small-t inhibits **large T antigen**-dependent replication. However, *in vivo*, small-t enhances SV40 DNA replication. Infection experiments also indicate that small-t stimulates progression of permissive monkey cells - but not non-permissive rodent cells - from the G_0/G_1 to the S phase of the cell cycle, presumably leading to an optimal intracellular environment for viral replication. In rodent experiments, small-t mutants preferentially transformed rapidly proliferating lymphoid cells and appeared unable to transform cells that normally have a low mitotic rate, such as mesothelial cells. In these experiments, we also found that wild-type SV40 induces **mesotheliomas** in hamsters. Subsequently, we have found that more than 60% of human mesotheliomas contain and express SV40-like sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2400-02 PB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The role of subclinical infection and cytokines in preterm parturition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberto Romero, M.D.	Head	PB, NICHD
Others:		
Heriberto Araneda, MT	Visiting Assoc. Fellow	PB, NICHD
Ricardo Gomez, MD	Visiting Fellow	PB, NICHD
Hector Marcelo Ramirez, MT	Visiting Assoc. Fellow	PB, NICHD

COOPERATING UNITS *(if any)*

Department of Obstetrics and Gynecology, Wayne State University (David Cotton, MD, Yoram Sorokin, MD, Stanley Berry, MD, and Ruben Quintero, MD)

LAB/BRANCH

Perinatology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, Detroit, Michigan 48201

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to define the role of ascending intrauterine infection in the etiology of preterm labor and delivery, to elucidate the cellular and molecular mechanisms signalling preterm parturition in the setting of infection and to apply this knowledge to improve the diagnostic and therapeutic approach to preterm labor.

The laboratory concluded the following studies:

1. Frequency and clinical significance of microbial invasion in patients with preterm premature rupture of membranes: 38% of women with preterm premature rupture of membranes have microbial invasion of the amniotic cavity. These patients had a shorter admission to delivery interval, a higher rate of preterm delivery and perinatal morbidity than patients with sterile fluid.

2. Rapid methods for the diagnosis of microbial invasion of the amniotic cavity: Amniotic fluid interleukin-6 determination was found to be the most sensitive test for the detection of microbial invasion of the amniotic cavity.

3. Antibiotic administration to women in preterm labor: The Branch Chief assisted the Extramural Branch of NICHD in the design, conduction and analysis of the largest randomized clinical trial conducted to date to determine the effect of antibiotic administration to women with premature labor. Antibiotics did not improve perinatal outcome.

4. Studies of microphage-derived cytokines and infection-induced preterm labor: The laboratory determined that: 1) a dramatic increase in maternal serum concentrations of TNF, IL-6 and IL-1 and in amniotic fluid concentrations of IL-6 and IL-1 alpha precedes infection-induced preterm labor and delivery in animals and 2) IL-1 and TNF have direct effects on myometrial cells and increase oxytocin induced uterine contractility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2401-01 PB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The role of subclinical infection and cytokines in preterm parturition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberto Romero, M.D.	Head	PB, NICHD
Others:		
Heriberto Araneda, MT	Visiting Assoc. Fellow	PB, NICHD
Ricardo Gomez, MD	Visiting Fellow	PB, NICHD
Hector Marcelo Ramirez, MT	Visiting Assoc. Fellow	PB, NICHD

COOPERATING UNITS *(if any)*

Department of Obstetrics and Gynecology, Wayne State University (David Cotton, MD, Stanley Berry, MD, and Ruben Quintero, MD)

LAB/BRANCH

Perinatology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, Detroit, Michigan 48201

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to improve the diagnosis and treatment of fetal disease. Focus is placed on the prenatal diagnosis of congenital anomalies with non-invasive methods (i.e., high resolution ultrasound and Doppler color flow mapping).

The most significant contribution of the laboratory was the performance of the first successful endoscopic surgical procedure in the human fetus. Transabdominal thin-gauge fetoscopy was used to identify and ligate the umbilical cord of an acardiac twin. This procedure represents a new approach to the treatment of fetal disease. We anticipate that operative fetoscopy will have a role in the treatment of other conditions such as monochorionic twins discordant for congenital anomalies, twin to twin transfusion syndrome, pedunculated fetal tumors and other fetal and placental conditions amenable to surgical treatment.

The laboratory used diagnostic ultrasound to establish prenatal diagnosis of conditions heretofore undiagnosed (i.e., velamentous insertion of the umbilical cord). Intensive studies of the fetal hemodynamic system in patients with preterm labor and intrauterine growth retardation progressed. The goal of these studies is to develop non-invasive methods to assess cardiac function in the critically ill human fetus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00876-03 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determinants of Childhood Poison Ingestion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	K.F. Yu	Mathematical Statistician	PRB, DESPR, NICHD
	E. Toretsky	Statistician	BMS, DESPR, NICHD
	P. Moyer	Computer Specialist	CSB, DESPR, NICHD
	A. Trumble	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Medicine and Dentistry, University of Rochester (N.G. Hildreth, R.A. Lawrence, G.S. Liptak).

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.45	0.45	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Determinants of Childhood Poison Ingestion study investigates the relationship between social and behavioral factors and risk of childhood ingestions of household cleaning and home repair and maintenance products. The specific aims of the study are: 1) to determine the sociodemographic, predisposing, life events and situation, social network, behavioral, parent health protective behaviors for children, and household hazards that affect childhood poison ingestion; and poison ingestion; and 2) to examine the relative contributions of these factors to poison ingestion. Funding for this project was provided by the HBL, CRMC, NICHD to the Principal Investigator at the time Dr. Maiman was Associate Professor, Department of Pediatrics, University of Rochester.

001

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00877-03 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention and Education in CHD Reduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	R. DerSimonian	Mathematical Statistician	PRB, DESPR, NICHD
	E. Toretsky	Statistician	BMS, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Medicine, Northwestern University (P. Greenland) and School of Medicine and Dentistry, University of Rochester (N.G. Hildreth, C. Cox).

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.20	0.20	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Prevention and Education in Coronary Heart Disease (CHD) Reduction study investigates issues related to the future design and testing of behavioral and educational strategies to help children and adolescents reduce risk behaviors and acquire and practice positive health behaviors. Funding for this project was provided by the DHVD, NHLBI to Dr. Maiman at the time she was Associate Professor, Department of Pediatrics, University of Rochester and Co-Principal Investigator on the project. The PRB, DESPR is responsible for analysis and dissemination of study findings from the screening program related to functional, behavioral, and psychological outcomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2100-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prenatal Care Utilization and Adverse Health Behaviors During Pregnancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	J.D. Killen	Behavioral Scientist	PRB, DESPR, NICHD
	M. Augustyn	Staff Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Prevention Research Branch, DESPR

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(Formerly titled, Proximate Biobehavioral Risk Factors in Poor Pregnancy Outcomes)

This research involves a program of studies employing behavioral and social support interventions to improve early entry to prenatal care and adherence to the visit schedule and decrease adverse health behaviors during pregnancy. The studies will focus on adolescent females who are at high risk of obtaining late or no prenatal care and particularly vulnerable to negative health behaviors (e.g., smoking, alcohol abuse, poor nutritional choices, illicit drug use) that are associated with poor pregnancy and birth outcomes.

006

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2101-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Increasing Preventive Actions for Childhood Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.35	0.35	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major purpose of the Increasing Preventive Actions for Childhood Injury study is to develop and test an intervention to reduce parental unsafe practices for childhood injuries in the home. A multicomponent preventive intervention for pediatric health care providers and parents will be developed and evaluated.

008

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2102-02 PRB

PERIOD COVERED: October 1, 1992 through April 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Minimal Contact Smoking Cessation and Relapse Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.D. Killen Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Stanford University School of Medicine (S.P. Fortmann)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

010

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2103-02 PRB

PERIOD COVERED

October 1, 1992 through April 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Computer-Assisted CVD Prevention Intervention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Killen	Behavioral Scientist	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Stanford University School of Medicine (C.B. Taylor)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

01

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2104-02 PRB

PERIOD COVERED

October 1, 1992 through April 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

School Based Program for Prevention of Unhealthful Weight Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Killen Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Stanford University School of Medicine (C.B. Taylor)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Healthy Weight Regulation (HWR) study was conducted to evaluate the efficacy of a school-based curriculum designed to modify the eating attitudes and unhealthful weight regulation practices of young adolescent girls. Funding for this project was provided by NICHD at the time that Dr. Killen was Senior Research Scientist, Department of Medicine, Stanford University School of Medicine and Co-Principal Investigator on the project. The study consists of observations collected from 995 adolescent girls (11-13 years of age) who will be followed for a period of five years. The Principal Investigator (Dr. Killen) functioned as the Director of Research for the educational and behavioral component of the study and directed the teams which developed the intervention materials and instrument and data collection protocols.

012

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2105-02 PRB

PERIOD COVERED: October 1, 1992 through April 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
CVD Prevention Intervention Trial

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Killen	Behavioral Scientist	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Stanford University School of Medicine (J.W. Farquhar)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

015

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2106-02 PRB

PERIOD COVERED

October 1, 1992 through April 30, 1993

TITLE OF PROJECT *(50 characters or less. Title must fit on one line between the borders.)*

Combined Family and School Program for Disordered Eating/Obesity Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Killen Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.29	0.29	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major purpose of this proposal is to develop both school-based and family interventions to prevent adoption of unhealthful weight regulation strategies by adolescents.

This research builds upon an earlier study funded by NICHD at a time when Dr. Killen was on staff at the Department of Medicine, Stanford University School of Medicine. The proposed trial builds upon the foundation of the earlier study by 1) developing a special, intensive intervention for adolescents identified as "at-risk" for disordered eating and 2) the development of a family intervention component designed to teach parents to promote healthful weight regulation practices by their children.

016

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2107-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Children and Adolescent Trial of Cardiovascular Health (CATCH)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*
University of Texas at Houston (G.S. Parcel); University of Minnesota (C. Perry); University of California at San Diego (P. Nader); Tulane University (L. Weber)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.05	PROFESSIONAL 0.05	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is a test of the effectiveness of a multi-component, school-based intervention to modify CVD risk factors among preadolescent children. Blood pressure, blood lipids, body fatness, and behavioral risk factors are being assessed. The intervention includes curriculum, school environment, and family components. After an initial, 3-year pilot phase the study is now in the second year of a 4-year trial.

018

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2108-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Afro-American Adolescent Girls Growth and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Center for Health Promotion Research, School of Public Health, University of Texas Health Science Center at Houston (Wendell Taylor)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the study is to assess longitudinally among Afro-American girls the pattern and determinants of body weight, body composition, and aerobic fitness, and physical activity.

020

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2109-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
How Much Physical Activity Do Children Obtain During Physical Education Classes?

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Center for Health Promotion Research, School of Public Health, University of Texas Health Science Center at Houston (Wendell Taylor)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to determine the extent to which physical education in a regional sample of elementary and middle schools provides students with moderate to vigorous physical activity during PE classes.

022

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2110-02 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Preventing Problem Behavior Among Middle School Students

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Others:	Lois Maiman	Chief	PRB, DESPR, NICHD
	Kai Yu	Mathematical Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(Formerly titled Prevention of Alcohol and Other Drug Use Among Middle School Students)

The purpose of the study is to develop, implement, and test the efficacy of a multi-component program of school-based interventions in preventing problem behavior in a sample of middle school students. Interventions would be directed at the students to improve their resistance to problem behavior and toward the school environment and parent behavior to provide support for student prosocial behavior. The primary research question is, "What is the effect on the frequency of problem behavior among students participating in a special intervention that includes an orientation to middle school, nontraditional curriculum, and parent intervention compared to students in the comparison group?"

02-

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2111-02 PRB

PERIOD COVERED: October 1, 1992 through February 5, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: School-Based Prevention of Drinking and Driving

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Other:	M. Augustyn	Staff Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Prevention Research Branch, DESPR

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

026

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2112-02 PRB

PERIOD COVERED: October 1, 1992 through February 5, 1993

TITLE OF PROJECT (35 characters or less. Title must fit on one line between the borders.)
The Baltimore Project for the Prevention of HIV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Augustyn Staff Fellow PRB, DESPR, NICHD

COOPERATING UNITS (if any)

Baltimore City Health Department (J. Santelli); The Johns Hopkins University School of Hygiene and Public Health (D. Celentano)

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated.

027

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2113-02 PRB

PERIOD COVERED: October 1, 1992 through February 5, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Barriers to Prenatal Care

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Augustyn	Staff Fellow	PRB, DESPR, NICHD
Other:	L. A. Maiman	Chief	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

— (a1) Minors

— (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

028

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2114-01 PRB

PERIOD COVERED: October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

MD/Family Partnership: Education in Asthma Self-Management

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation*

PI: L.A. Maiman Chief PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Public Health, University of Michigan (N.M. Clark, D.W. Roloff, M.E. Hurwitz, M.A. Schork, M.H. Becker) and School of Medicine, Columbia University (D.R. Mellins, D. Evans)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The major purpose of the MD/Family Partnership: Education in Asthma Self-Management study is to develop and test an intervention based on theories of self-regulation of behavior in changing the diagnosis and treatment patterns and educational behavior of physicians who treat children with asthma.

029

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 2115-01 PRB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunization Among D.C. Infants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Others:	John Clemens	Chief	EB, DESPR, NICHD
	Jennifer Read	Senior Staff Fellow	EB, DESPR, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.15	0.15	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this program of research is to assess the determinants of non-immunization and to test the efficacy of interventions to improve immunization status of DC infants. Following a successful efficacy study, an large-scale effectiveness trial will be conducted.

03

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2116-01 PRB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Preventing Pregnancies Among Adolescent Girls in the District of Columbia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS (if any)

Howard University Medical Center (R. Jenkins, MD), Georgetown University Medical Center (J Cox, MD; P. Bush, PhD), Children's Hospital (K. Woodward, MD), DC Commission of Public Health (P. Sharps, PhD), DC Hospital (Maislyn Christie, MD)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this research is to test the efficacy of a program of intensive interventions to prevent adolescent pregnancies.

033

GPO 914-918

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